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ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FIRST
ANNUAL MEETING OF THE AMERICAN PHYTOPATHO-
LOGICAL SOCIETY, COLUMBUS, OHIO, DECEMBER
27 TO 30, 1939, INCLUSIVE

(NOTE: The arrangement of the abstracts is alphabetical by author or senior author.)

A New Strain of the Tomato Leaf Mold Fungus Cladosporium fulvum. L. J. ALEXANDER. Several workers have reported that the tomato species, *Lycopersicon pimpinellifolium*, is highly resistant to the tomato leaf-mold fungus, *Cladosporium fulvum*. Recently, the new variety Globelle, developed from crosses between *L. pimpinellifolium* and varieties of the domestic species suddenly became susceptible. Previously, Globelle had shown as high a degree of resistance as *L. pimpinellifolium*. Investigations that followed have shown that the difference in resistance is due to a new strain of the leaf-mold fungus. In cross-inoculation experiments, plants of the varieties Globe and Globelle were uniformly susceptible to spores of the fungus collected from diseased Globella leaves. However, when plants of the two varieties were inoculated with spores collected from diseased Globe leaves, secured from a location remote from where the new strain of the fungus was present, the Globelle plants were highly resistant and the Globe plants susceptible. Inoculation experiments also have shown that *L. pimpinellifolium* is susceptible to the new strain of *C. fulvum*.

Bacterial Stalk Rot of Field Corn Caused by Phytonomas lapsa, n. sp. P. A. ARK. Bacterial stalk rot of field corn occurred in epidemic form in several counties in California in 1937. It resulted from the use of contaminated seed or from survival of the organisms in the soil. It is a parenchymatous disease. Under favorable conditions (high humidity and high temperatures) a rapid rotting of the leaves and the stalk occurs, often causing the plants to fall to the ground. The pathogen was isolated from the external parts and from the alimentary organs of *Diabrotica* beetles. The organism is a short bacterium ($1.55 \mu \times 0.56 \mu$), motile by 1 to 4 polar flagella. It produces fluorescence in Uchinsky's, Fermi's, and Cohn's solutions. Acid, but no gas, is produced from dextrose, sucrose, maltose, lactose, glycerine, arabinose, xylose, galactose, raffinose, and mannitol. The name, *Phytonomas lapsa* is proposed, the Latin word *lapsus*, meaning falling, descriptive of one of the symptoms of the disease. The disease is controlled by dusting the seed with Semesan Bel. Of many species of plants inoculated with *Phytonomas lapsa, n. sp.* only sugar cane seedlings developed a disease similar to that of corn.

Relation of Reducing Substances to Longevity and Virulence of Phytopathogenic Bacteria. P. A. ARK. Vitamin C (1:200 to 1:1,000,000), cysteine (1:1,000), glutathione (1:1,000), pyrogallol (1:1,000), resorcinol (1:10,000) and tannic acid (1:100 to 10,000) prolonged the life of *Erwinia amylovora*, *Phytonomas malvacearum*, *Ph. pisi* and *Ph. stewartii*, when these materials were incorporated into potato-dextrose-peptone agar on which the pathogens were grown. The higher the concentration of the vitamin C and other reducing substances the longer the bacteria can live on the medium without subculturing. Moreover, this property is retained by the organisms for some time when they are transferred into media without the reducing substances. Thus, *E. amylovora* lived 6 months instead of 2 weeks on the potato-dextrose-peptone agar when transferred to this medium after 2 months on media containing vitamin B (betoxin, 15 mg. per liter of media) or resorcinol (1:10,000). Virulence of *E. amylovora* diminished when grown without subculturing from 1 to 4 months on the potato-dextrose-peptone agar plus the reducing agent. Virulence returns to normal very promptly, even after the first transfer to a medium without any reducing agent or a medium of a very low reduction potential.

Fusarium Wilt of Cotton and Tobacco Apparently Caused by the Same Organism. G. M. ARMSTRONG. Isolates of *Fusarium* spp. obtained from wilting cotton and Burley tobacco in South Carolina have been used in cross inoculations on the susceptible varieties Farm Relief (cotton), Burley 5 (tobacco), and the resistant tobacco varieties, Burley 31 and Gold Dollar. A *Fusarium* obtained from wilting tobacco in Kentucky also was used with the tobacco varieties. Inoculations have been conducted in water-cultures in the greenhouse and in pots of soil out of doors. The wilting of the susceptible cotton and tobacco varieties in water-culture after inoculation with the S. C. cotton fungus varied from 87 to 100 per cent and with the S. C. tobacco fungus, 87.8 to 100 per cent. Seventeen per cent of wilting occurred with one resistant variety of tobacco inoculated with the cotton fungus. No external symptoms of wilting occurred in either resistant tobacco variety grown in soil, though the fungus was recovered from some plants inoculated with

each isolate. Wilting of the susceptible variety of tobacco in water culture was slightly more than twice that in soil. These results indicate that *Fusarium vasinfectum* and *F. oxysporum* v. *nicotianae* are the same. (Cooperative investigations, South Carolina Agricultural Experiment Station and Division of Cotton and Other Fiber Crops and Diseases, U. S. D. A.)

Studies of Olpidium trifolii and Urophlyctis trifolii on White Clover in Louisiana. R. E. ATKINSON. Leaf curl of *Trifolium repens*, caused by *Olpidium trifolii*, was found in early February, 1939, in almost every clover patch in the vicinity of Baton Rouge, Louisiana. A month later a gall, caused by *Urophlyctis trifolii*, was observed on the same host in 2 low areas that were inundated for several hours after every heavy spring rain. This is the first report of these 2 organisms in the United States. Ever since the original descriptions of these 2 fungi, there has been considerable uncertainty as to whether or not they were identical. The writer's studies, however, indicate that they are different. *Olpidium trifolii* produces zoosporangia with 1 or more exit tubes. Both resting spores and zoosporangia are characteristically found in the epidermal cells. Resting spores are from 15 μ to 40 μ in diameter. Resting spores of *Urophlyctis trifolii*, ranging from 30 to 50 μ in diameter, are formed in subepidermal chambers by a definite rhizo-mycelium of turbinate cells and slender strands. The so-called haustoria form a crown about the top of the young resting spores. When the spores mature, the "haustoria" disappear, leaving 9 to 15 pits in the external wall, as is true of *Urophlyctis alfaiae*, also.

Pathogenicity and Hosts of the Fly-Speck Fungus of Apple. R. C. BAINES. The fly-speck fungus *Microthyriella rubi* has been collected on the following hosts: *Acer saccharum*, *Quercus alba*, *Rubus allegheniensis*, *Sassafras variifolium*, *Salix nigra*, *Smilax hispida*, *Staphylea trifolia*, *Gleditsia triacanthos*, *Rhus glabra*, and *Xanthoxylum americanum*. Mature ascocarps, asci, and ascospores from each host were morphologically similar. Ascospores were found to mature during the first part of June at Lafayette, Indiana. The fungus was isolated in single-spore cultures from the first 7 hosts listed above. Cultures from all 7 appeared very similar on potato-dextrose agar. The fungus grows slowly and produces a grey, compact colony on most agar media. Growth was obtained between pH 1.8 and 8.2 on oatmeal agar. Growth occurred over the temperature range, 5-27° C., best growth appearing between 15-24° C. Attempts to induce sporulation in culture failed. Immature apple fruits were inoculated with mycelium from cultures isolated from the first 5 above-named hosts. Typical fruiting bodies and symptoms of fly speck were obtained in each case. The fungus differs markedly in symptoms produced on apple and in cultural characteristics from the sooty blotch fungus *Gloeodes pomigena*.

Acquisition and Transmission of Viruses by Dodder (Cuscuta subinclusa.) C. W. BENNET. Dodder (*Cuscuta subinclusa*), growing on curly-top-infected beet or Turkish tobacco plants appears to acquire concentrations of virus equal to those of the host plant. When stems of dodder were trained from diseased beets and established on healthy beet or Turkish tobacco plants the curly-top virus was transmitted to from 2 to 5 per cent of the plants. Where no transmission occurred the virus was lost from the dodder within a few days following breaking of connection with infected plants. Dodder became infected with cucumber mosaic virus, retained the virus indefinitely on immune plants, and transmitted it to more than 90 per cent of Turkish tobacco and *Nicotiana glauca* plants on which infected stems became established. No virus was obtained from juice of dodder growing on beet plants affected with beet mosaic or from juice of dodder growing on Turkish tobacco plants affected with common tobacco mosaic. By means of dodder the virus of cucumber mosaic is easily separated from a mixture of cucumber-mosaic virus and tobacco-mosaic virus in Turkish tobacco. Transmission of the virus of cucumber mosaic from Turkish tobacco to Turkish tobacco and *N. glauca* was obtained also, using *C. californica*.

Mechanical Transmission of Aster-yellows Virus to Leaf Hoppers. L. M. BLACK. The aster-yellows virus has been mechanically transferred from viruliferous to non-viruliferous aster leaf hoppers (*Macrostelus divinus*) at 0° C. Dilutions of juices from macerated viruliferous leaf hoppers as high as 1:100 in 0.85 per cent NaCl solutions are infectious when injected into nonviruliferous leaf hoppers by means of capillary glass tubes. As high as 40 per cent of the inoculated insects become infective for plants after an incubation period of from 2 to 6 weeks. Leaf hoppers, infected mechanically, appear to transmit virus to healthy aster plants as efficiently as insects that become viruliferous through feeding on diseased plants. The successful mechanical transfer of virus from insect to insect has made possible a study of virus multiplication in this vector. Non-viruliferous leaf hoppers were fed 2 days on diseased aster plants and then maintained on rye, which is immune from aster yellows. No virus could be detected in such insects

during the first few days after they had fed on diseased asters. Later, however, juice from such leaf hoppers was infectious at dilutions up to 1:100 when injected into non-viruliferous insects. This is considered good evidence of multiplication of aster-yellows virus in its insect vector.

Cytological Studies of Sporidial Fusion in Ustilago zae. DONALD H. BOWMAN. Sporidial fusion in paired monosporidial cultures originating from single chlamydospores of *Ustilago zae* was found to occur readily, although not abundantly, in cultures of very low nutritive value after a period of 15 to 20 hours, depending upon the temperature at which the cultures were incubated. Initiation of the dikaryophase did not occur in any fixed manner, but was effected by conjugation of 2 compatible haploid cells, either sporidia or cells of 2 haploid hyphae resulting from sporidia. Nuclei from the haploid cells became paired in a fusion cell, which gave rise to the dikaryotic hypha. Such hyphae continued growth throughout the experiment without reverting to the production of sporidia. The end cells of the dikaryotic hyphae absorbed and retained the various dyes used much more readily than did the older portions of the hyphae and the sporidia. Apical cells of these hyphae uniformly contained one pair of nuclei each. The older cells contained well-defined nuclei either singly or in pairs or were partly or entirely devoid of cell contents. Cells of fused sporidia frequently contained a single nucleus or were either partly or entirely empty.

The Boron Deficiency Disease of Apple. A. B. BURRELL AND F. H. LEWIS. Numerous terms for the different symptoms are in use. Confusion would be avoided if all but the following were eliminated: internal and external cork of fruit; incipient dieback, dieback, rosette, (and possibly internal bark necrosis). Drouth indirectly was responsible for a 1939 outbreak of cork in New York and New England. Borax applied to the soil gave control in the 3 major apple-producing regions of New York. Borax soil applications, in the spring of 1937, were still effective throughout 1939. Part of the boric acid injections made in late summer of 1936 had become ineffective by 1939. A borax soil application on June 30 when some cork already showed, largely prevented late-season development, which was abundant in checks. The effectiveness of borax added to orchard sprays remains in doubt. Trees treated with Chilean nitrate of soda have not shown significantly less cork than those receiving other nitrogen carriers. Six annual applications of manure did not prevent internal cork. Extreme soil acidification with sulphur applied from 1927 through 1937 failed to give cork control in 1939. In commercial practice, minor boron foliage injury occasionally results from excessive soil application to young replants and from careless concentrated application near tree trunks.

Sulphur as a Protectant of Cereal Crops. KARL D. BUTLER. Sulphur dusting of cereal crops on a field scale has been carried on in New York state for the past 3 seasons. Through the use of a power duster mounted on a pick-up truck and equipped with a 40-foot boom, effective protection has been obtained. Epiphytotics of *Puccinia rubigo-vera tritici*, *P. coronata* and *Erysiphe graminis hordei* have been checked with 2 to 5 applications of sulphur at the rate of 30 pounds an acre per application. In 1938 and 1939 satisfactory protection of wheat from *P. rubigo-vera tritici* was accomplished with but 2 well-timed applications. Timing of applications was facilitated by studies on the dissemination of urediospores by means of weather-vane spore traps. Increased yields in bushels per acre ranged up to 10.45 of wheat, 25.53 of oats and 5.36 of barley in 1937; 12.56 of wheat, 28.81 of oats, and 10.23 of barley in 1938; and 8.38 of wheat, 6.90 of oats, and 6.85 of barley in 1939. Reduced yields due to injury caused by the equipment in wheat fields were found to be slightly less than one bushel per acre.

Cross Inoculations with Loose Smut of Wheat. RALPH M. CALDWELL AND L. E. COMPTON. Seven collections of loose smut, *Ustilago tritici*, obtained from individual soft wheat varieties in Indiana, Ohio, and Illinois, in 1938, were tested for pathogenicity on 7 soft wheats, (Kanred-Gipsy, Purdue No. 4, Nabob, Forward, Trumbull, Hussar, Kawvale) showing field resistance, and 2 (Wabash, Purdue No. 1) showing high field susceptibility. Inoculations, by the part-vacuum technique, produced over 75 per cent infection in highly susceptible varieties. Evidence was secured of a high degree of host specialization of the loose-smut fungus as it occurs on soft wheats in this region. The field-susceptible varieties, Wabash and Purdue No. 1, were heavily infected when inoculated with spores from the same 2 varieties, respectively. However, in cases where either variety was inoculated with smut from the other variety, a high degree of resistance, or immunity, was demonstrated. Kanred-Gipsy, a variety previously found resistant to both natural and artificial infection, proved highly susceptible to 1 collection from Ohio. Purdue No. 4 proved highly resistant to certain collections but susceptible to others. Kawvale and Trumbull were resistant to all collections, while Nabob, Forward, and Hussar, each, produced a few infected plants from certain collections. (Department of Botany, Purdue

University Agricultural Experiment Station, Lafayette, Indiana, and Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

The Effect of a Diseased Cotton Seed on an Adjacent Healthy Seed. K. STARR CHESTER AND GERTRUDE TENNYSON. The question of whether a diseased cotton seed jeopardizes the success of adjacent healthy seed was tested by direct observation of the success of healthy seed in the presence of diseased seed under greenhouse conditions and by analysis of greenhouse and field data in the light of the mathematical probability of successful development of healthy seed alone or in the presence of diseased seed. The diseased seeds were infested with various amounts of *Glomerella gossypii*, *Fusarium moniliforme*, *Sclerotium bataticola*, *Bact. malvacearum*, and other organisms. The results indicated that the stated hazard is slight or negligible under a variety of environmental conditions, implying that the beneficial effect of "Ceresan" is principally on the treated seed itself, and that external factors, principally *Rhizoctonia*, are of much greater importance in influencing the success of seedlings than infestation among the seed.

Disease Resistance in the Genus Nicotiana. E. E. CLAYTON AND H. H. FOSTER. Over 1,000 collections of *N. tabacum* ($n=24$) from all known areas of occurrence have been tested for resistance to blue mold (*Peronospora tabacina*), black root rot (*Thielaviopsis basicola*), wildfire (*Bacterium tabacum*), bacterial wilt (*Bacterium solanacearum*), root knot (*Heterodera marioni*), and mosaic. A high degree of resistance was found for black root rot and mosaic, but only moderate to slight resistance for root knot, wildfire, blue mold, and wilt. Wilt, root-knot, and blue-mold resistance was recessive and conditioned by multiple factors. Some 30 other *Nicotiana* species have been studied with respect to disease resistance. Some of the more promising of these are, *N. debneyi* ($n=24$), which appears immune from blue mold and black root rot, and resistant to wildfire; *N. glauca* ($n=12$) immune from black root rot and highly resistant to mosaic, root knot and wildfire; *N. repanda* ($n=24$) highly resistant to wildfire, black root rot, and root knot. Smiths allo-polyloid (*N. tabacum* \times *N. glauca* - $n=36$) shows resistance to root rot and root knot. These, and other species of *Nicotiana*, offer a reserve of resistance approaching immunity for all diseases considered, excepting bacterial wilt.

Sweet-potato-storage House Fumigation. HAROLD T. COOK AND T. J. NUGENT. Formaldehyde, a formaldehyde-sulphur dioxide mixture, chloropierin, ammonium hydroxide, carbon tetrachloride, and ethylene dichloride were compared as fumigants for sweet-potato-storage houses. The materials were tested on spores from pure cultures of *Rhizopus nigricans* and *Ceratostomella fimbriata* applied to wooden blocks, suspended in 1-liter flasks. After the fumigants were added the flasks were sealed with glue-coated paper for 24 hours. The quantity of formaldehyde usually recommended (3 pt. to 1000 cu. ft.) was found to be about 4 times the amount needed for sterilization. Fumigation tests for shorter periods indicated that the fungi were killed within 2 hours. Larger quantities of the fumigants were required for sterilization when the storage chambers were partly filled with pieces of wood. Chloropierin was effective against both fungi, even at concentrations as low as 1/16 lb. in a moist atmosphere, but was only partly effective, even at higher concentrations, in a dry atmosphere. Ammonium hydroxide and the formaldehyde-sulphur dioxide mixture were not so effective at low concentrations as were formaldehyde and chloropierin. Carbon tetrachloride and ethylene dichloride were entirely ineffective against *Rhizopus*, even when 12 pints to 1000 cubic feet were used.

Preliminary Serological Studies of Phymatotrichum omnivorum. R. W. CUMLEY AND G. W. GOLDSMITH. A preliminary serological study was carried out to determine the relationship of the cotton root-rot fungus, *Phymatotrichum omnivorum*, to the various members of the different groups of fungi. Extracts of freshly collected or cultured species were compared with the root-rot fungus in the precipitin and the complement fixation tests. Results indicate that the cotton root-rot fungus serologically is more closely related to the various Gasteromycetes than to the other fungi tested.

Organic Seed Protectants for Lima Beans. H. S. CUNNINGHAM AND E. G. SHARVELLE. Damping-off in most seasons constitutes a major problem in the chief Lima-bean growing areas of the United States. No chemical seed treatment has yet been found that is entirely suited to Lima beans. Organic mercurials usually stunt the plants throughout their lifetime, while copper oxides in many instances harden the seed coat, making it difficult for the plumule to break out. Preliminary laboratory and greenhouse experiments indicated that 2 synthetic organic chemicals developed by the Crop Protection Institute were efficient, noninjurious seed protectants for combating damping-off of Lima beans. The laboratory and greenhouse findings were substantiated by field experiments conducted on Long Island during the past season, where seed treatment with the new materials No. 120, No. 98, and New Improved Semesan Jr. in that order resulted in

significant reduction of seed decay of the Lima beans. Graphite seed treatment reduced drilling friction, and that in turn appeared to reduce cracking of seeds and subsequent decay in the soil.

A Method for Testing the Pathogenicity of Actinomyces Isolates. PHARES DECKER. Plants were grown from scab-free, cold-formaldehyde-treated tubers in sterilized silt-loam soil in 6-inch clay pots, each fitted with a 6-inch clay saucer. The isolates were increased on potato-dextrose agar in Petri dishes and washed from the plates into the sterilized soil over the seed piece and covered 2 inches deep. The greenhouse, equipped with a painted concrete floor and metal-leg benches with wooden tops, was fumigated with formaldehyde gas and sterilized with a 2 per cent solution of carbolic acid before the pots were moved in the house. The water was applied to the pots by means of the 6-inch clay saucer placed under each pot to avoid overhead splashing. During the past year, of the 225 isolates tested, each replicated 4 times, 35 were strongly pathogenic, 43 were weakly pathogenic, while 157 were nonpathogenic under the conditions tested. The tubers from 300 control pots placed at random among the pots containing the scab inoculum were absolutely scab-free.

The Relation of Stomata to Wildfire Infection. STEPHEN DIACHUN. The amount of wildfire on inoculated leaves of greenhouse or field-grown tobacco plants was found to be dependent on the stomatal condition of the leaves at the time of inoculation. During the day stomata are usually open and tender leaves of vigorous plants atomized with a bacterial suspension become severely infected if the nozzle of the atomizer is held about 2 inches from the lower surface of the leaves. At night or in artificial darkness stomata are usually closed or nearly so; they also are apt to be closed during the day on leaves that are wilted, excessively shaded, or turned up so that the lower surface is exposed to the sun. Under any of these conditions, when stomata are closed, leaves atomized with *Bacterium tabacum* develop little infection. During some rainstorms stomata were open, and during others they were closed, depending perhaps on the light intensity. It is suggested that natural infection by bacteria carried in wind-blown rain may occur only when stomata are open.

Inoculation Experiments and Reaction of Inbred Lines of Corn to Ustilago Zeae. JAMES G. DICKSON AND DONALD H. BOWMAN. Inoculation studies with *Ustilago zeae* have been conducted during the past 2 seasons. A spore suspension in 0.8 per cent solution of rosin-fish-oil spray soap appeared applicable to large-scale inoculation experiments and gave good differentiation for smut reaction on inbred lines of corn. Six such lines, differing in resistance to smut, were inoculated at 4 stages of plant development in 1939. The soap solution alone increased smut infection an average of 10.2 per cent over the controls. The suspension of chlamydospores in the soap solution increased smut infection an average of 39.6 per cent over the controls. The range in averages for the individual lines was from 11.2 to 69.7 per cent increase over the controls. The smut reaction of the individual lines in the inoculation experiments for the 2 years was in fair agreement with that resulting from natural infection. (Cooperative investigation between the Wisconsin Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Modifications in Cells of Plants Affected by Virus. JEAN DUFRENOY. I. The inhibition of terminal growth at root tips of sugar beet affected by *Jaunisse* (a virus disease in northern France and Belgium) results in many mitochondria differentiating into "starch-storing" amyloplasts in postmeristematic cells, where homologous cells of sound beets show only rod-like "inactive" mitochondria. The same response has been observed in beets affected with curly top.

II. Enhanced respiration in virus-affected cells may shift the pH of the vacuolar solution upwards, as organic acids are oxidized to CO₂. The lowering of the acidity, together with altered viscosity resulting from accumulation of tannin in the vacuolar solution, causes calcium oxalate to crystallize as large tetrahedric crystals, often clumped together as, for example, in cells of Up-to-Date potato leaves, along the necrotic perivascular lesions caused by Virus Y.

III. Chloroplasts in affected leaves are more apt to swell, when sections of fresh tissues are immersed in hypotonic solutions: chlorophyll may migrate away from swollen chloroplasts, to be absorbed in oil inclusions.

Reactions of Cells of Sugarcane Stalks to the Red Rot Fungus, Colletotrichum falcatum Went. JEAN DUFRENOY AND C. W. EDGERTON. Following inoculation, spores lodge in the pitted vessels and germinate. Mycelium grows through pits into the living cells of the parenchyma and comes into close contact with the nuclei. Vacuolation is induced and numerous oil droplets along the cytoplasmic strands outline the vacuoles.

In susceptible varieties such as C.P. 807, more cells become heavily infected as the infected cells die and the hyphae are outlined by rows of droplets of vacuolar material showing staining reactions of phenolic compounds. In resistant varieties such as Co. 281 phenolic compounds are evident throughout the vacuolar system of infected cells and the mycelium becomes imbedded in the reactive products of the cell. The red pigment produced by the infected tissues diffuses out into the adjoining cells and is absorbed by the lignified spirals of the vessels. From sections of red-rot lesions, treated with 0.25 per cent NaOH, a yellow solution is obtained, which oxidizes and turns brown on contact with the air.

The Influence of Ultraviolet Irradiation on the Pathogenicity of Phytomonas tumefaciens. B. M. DUGGAR AND A. J. RIKER. In this work monochromatic ultraviolet of wave length 2650 Å was employed, and suspensions of the bacteria were exposed for a time to intensities sufficient to give about 99 per cent killing, i.e., 1 percentage survival. Four strains of the organism were selected; one of these characteristically virulent (gall-producing), one attenuated by chemical treatment, and two strains partially attenuated by the same type of treatment. Of these two last, one was losing and one recovering gall-producing capacity. Gall production of all treated and control suspensions was tested by inoculation into young tomato plants, with periodic observation and measurement of the gall number and size. About 50 colony isolates of each exposure were used, and for each isolate 10 punctures were made; the control was similarly used. The present status of the results point to the following: Neither was the virulent strain nor the avirulent one materially affected in pathogenicity by irradiation. The partly virulent strains, on the whole, gain in pathogenicity as a result of irradiation. The survivors of irradiation-treated isolates exhibit a greater range of variability than the corresponding controls.

Root Girdle Caused by High Wind. E. L. FELIX. Root girdle, characterized by marked constriction of the primary root near the soil surface, frequently causes heavy losses in small seedlings of beets, carrots, lettuce, and spinach in Western New York, especially on muck soil. In lettuce, root girdle (previously incorrectly called stem-girdle) commonly destroys plants one-fourth or more grown. Lettuce suddenly turns yellow, wilts, easily breaks off at the constriction, and is sometimes mistaken for cutworm injury. Reddish to reddish-brown vascular discoloration sometimes originates below the girdle and extends upward to it, seldom above. Leaves of girdled lettuce sometimes are inclined in one direction and the roots in the opposite, presenting a one-sided appearance. That high wind is the primary cause of root girdle is indicated by observations over a 15-year period. Root girdle occurs only following high wind, usually 2 to 4 days later, and in wind-swept portions of the field. It appears and disappears in large part suddenly and simultaneously. Occurrence is of short duration, the majority of affected plants dying in 7 to 10 days. Leaf inclination in girdled lettuce tends to be leeward. Associated bacteria and *Botrytis* fail to reproduce typical root girdle and are secondary invaders. The manner of girdling appears to be mechanical injury, desiccation, and shrinkage of tissues.

Variation in Dothiorella ulmi, the Elm Cephalosporium. LAWRENCE M. FENNER. Variation in cultural characters in the elm die-back organism, *Dothiorella ulmi*, has been observed. Sixteen isolates from widely distributed points in the eastern United States have been studied by single-spore methods and sector subcultures. Three phenotypic groups, A, B, and C, were noted. The range of variation was greatest in group A. A few forms have been considered relatively stable. Certain phenotypes are reported as new and undescribed in the literature. In a study of more than 2,000 single-spore colonies and their numerous subcultures, a close relationship was recognized among the phenotypes of the 3 groups.

The Thixotropic Character of the Tobacco-Mosaic-Virus Protein. VERNON L. FRAMPTON. Three hundred feet of 16 mm. Kodachrom photographed through crossed polaroid plates. Interesting features include:

1. An indication of the sol-gel transformation time.
2. The solid character of the gel depicted in a rotating test tube. By way of contrast, debris suspended in water does not rotate with tube, whereas the virus protein gel does.
3. The evidence that the Brownian movement is greatly curtailed is seen in the persistence of the orientation of the anisodimensional aggregates—an orientation induced by a sphere falling through the gel.
4. Vortex motion in true solution is contrasted with the behavior of the virus protein gel under comparable conditions.

5. Demonstration of the gel character of the protein-water system by metathesis of BaSO_4 in the form of what appears a solid vertical rod in the center of the gel.
6. The swastika-shape cross of isocline.
7. The spontaneous repair of structure broken by forced flow.
8. The elastic character of the gel as shown by the very strongly damped harmonic motion of the gel mass as it comes to rest after having been disturbed.
9. A comparative study of the viscosity and diffusion of the protein-water system and of glycine.

Some Factors that Affect the Spray Program in the Control of Cedar-Apple Rust Fungi on the Apple. J. M. HAMILTON AND L. O. WEAVER. Studies on potted apple trees in a controlled environmental set-up indicate that fungicides are effective only against infection from the sporidia of the cedar-rust fungi over the area actually covered. Liquid lime-sulphur 1-50 is effective when applied 8 hours after the sporidia are *in situ*, but the wettable sulphurs do not inhibit infection at 4 hours. Foliage infection takes place in 6, 4, 2, and 5 hours at temperatures of 9, 13, 17, and 21 degrees C., respectively, but not at 25°. Sporidia may remain viable on foliage without moisture for 24 hours; but there is a marked reduction in the amount of infection after 4 hours. Experiments conducted on dwarf Wealthy and Delicious trees with the apple and quince rusts suggest that fruit parts may be susceptible any time after the opening of the fruit cluster. Prevention of sepal infection is important. The quince rust fungus causes heavy fruit drop, not usually apparent when infection takes place during or soon after bloom. The fruit is most heavily infected at 4 to 10 mm. diam., but it is susceptible at 52 mm. Fruit infection has been obtained in 5 and 12 hours at 16° C. with both rusts.

Methods for Determining the Effectiveness of Fungicides Against Apple Scab and the Cedar-Apple Rust Fungi. J. M. HAMILTON AND L. O. WEAVER. The technique of spraying fungicides on potted apple trees, washing them on a turn table, and testing the fungicidal residue by subjection to an infection period against the spores of *Venturia inaequalis* in a temperature-humidity controlled chamber has been improved upon. Regulated spraying of trees on the turn table, together with modified sulphur and copper analytical methods, has made possible accurate evaluation of fungicides and a more valuable interpretation of data from analyses of fungicidal residues taken in the field. The use of the sporidia of *Gymnosporangium juniperi-virginianae* is preferable to the spores of *V. inaequalis*. A precision laboratory sprayer—a material but limited aid in preliminary fungicidal studies—has been adopted, with the above set-up, for making exact depositions of fungicides or spore suspensions on foliage. Half of each leaf can be sprayed and the other half left as a check. The initial deposit is most accurate and can be tested with or without washing. For example, 30 to 40 gamma of sulphur per sq. in. are required to give protection against sporidia of *Gymnosporangium juniperi-virginianae*, whether it is an initial deposit or residue obtained by washing.

The Dissemination of Yellow Dwarf of Potatoes and Its Leaf Hopper Vector, Aceratagallia sanguinolenta. E. D. HANSING AND VERNON L. FRAMPTON. The incidence of yellow dwarf in potato fields indicates that its leaf-hopper vector migrates in obedience to the laws of diffusion. Data obtained from samples of tubers taken at various distances from adjacent meadows in 2 fields of the variety Rural, and indexed in the greenhouse,

fit the curve $-\log \frac{I}{I_0} = Rd$ where I is the percentage of infection at the distance d from the meadow, I_0 is the "saturation value" in per cent at the edge of the field, and R is a constant characteristic for the particular observation and represents a composite of various factors, such as population density, proportion of viruliferous insects, prevailing winds, food supply, etc. Values for $\log I_0$ and for R were 1.78 and .019, respectively, in the one case and 1.32 and .015 in the other. Conclusions that seem reasonable on the basis of these data are: (1) The migration of the insects parallels that of a diffusing substance; (2) The principal vector occurs naturally outside the field in question; (3) The spread from potatoes infected during the current season is a minor factor in the dissemination of the disease.

Insects in Relation to Root Rot and Basal Stem Rot of Cereals. E. W. HANSON AND J. J. CHRISTENSEN. Studies during recent years have shown that several species of insects, most of which are not yet definitely identified, attack the basal parts of wheat, barley, and several wild grasses, furnishing avenues of entrance for fungi, in some cases filling the culms with frass, which is a good medium for rapid growth of fungi, and predisposing the plants to root rot and basal stem rot. Infestation of this type has been common throughout the spring-wheat area for several years, the amount varying with the locality and year. In 1938 and 1939, from 70 to 90 per cent of the plants of certain varieties were infested. The durum variety Mindum was extremely susceptible, bread

wheats were moderately susceptible, and oats was virtually free from infestation. Many grasses, including *Agropyron repens*, *A. smithii*, *A. cristatum*, *Bromus inermis*, and *Echinochloa crusgalli* also were infested and predisposed to rots. *Helminthosporium* spp. and *Fusarium* spp. were isolated most commonly. The recognition, selection, and development of varieties resistant to root and foot rot must take into consideration susceptibility or resistance to the predisposing insects and the interrelationships between them and the rots. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Effect of Fertilizers on the Development of Bunt of Wheat. E. W. HANSON AND I. W. TERVET. It has been difficult to obtain consistently satisfactory infection with bunt at University Farm, St. Paul, Minnesota. Therefore, a study was made of factors affecting its development. As fertilizers have been reported to be important, potash, ammonium phosphate, ammonium sulphate, cyanamid, acid phosphate, and urea were added to the soil separately at the rates of 250, 200, 200, 200, 150, and 130 lb. per A., respectively, in 2 applications—the first, 10 days after planting and the second, 13 days later. Seed of 2 varieties, Ceres and Thatcher, was inoculated with a composite sample of chlamydospores of several physiologic races and collections of *Tilletia levis* and *T. tritici*. The experiment included 12 replications in 1938 and 4 in 1939. The relative amount of bunt was high (41.6 per cent on Ceres) in 1938 and very low in 1939 (3 per cent on Ceres), but in neither year was there any correlation between the amount of bunt and fertilizers applied. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

A Fungous Parasite of the Pine Bark Beetle. J. G. HARRAR AND J. G. MARTLAND. During the spring of 1939, collections of bark of *Pinus echinata* from eastern Virginia were found infested with the larvae of the pine bark beetle, *Dendroctonus frontalis*, many of which were dead or dying from what appeared to be the action of some parasite. Upon examination, large numbers of the affected larvae were found infested with both fungi and nematodes. Larvae from a number of collections were plated out, and a similar fungus was isolated from each of several larvae from the different collections. A study of monosporous cultures of the fungus has resulted in its tentative identification as a species of *Beauveria*. Results of subsequent experiments have shown that this organism may live as an insect parasite.

A Wilt of Tree Paeonia (Paeonia moutan). M. R. HARRIS. A fungus disease of tree paeonia has been observed in the San Francisco Bay region since 1932. On old plants it causes a wilting and sudden killing of the current season's growth. The first evidence of the disease is a watersoaked lesion that rapidly turns a light brown, encircles the stem, and causes the growth above the lesion to wilt and collapse. Losses are most severe during the flowering season. If allowed to go unchecked young shoots wilt successively as they appear and the plant eventually dies. Isolations and pathogenicity studies have shown that 2 species of *Coniothyrium* cause the disease. One of these is believed to be *Coniothyrium fuckelii*. Cross inoculations or roses have shown that both fungi can parasitize roses and paeonias. Histological studies show that the fungi are largely confined to the cortical region of the stems. Effective control in a commercial nursery has been accomplished by pruning out infected stems and spraying with ammoniacal copper carbonate.

Transformation of Haustoria and Hyphal Tips of Puccinia graminis tritici into Spore-like Bodies. HELEN HART AND J. LEWIS ALLISON. Haustoria of certain races of *Puccinia graminis tritici* may be transformed into urediospore- or teliospore-like bodies within host cells and the hyphal tips of the intercellular mycelium may develop into teliospore-like bodies at a temperature of 29° C., or higher, in seedlings of certain wheat varieties. External evidence of the transformation is the browning of host tissues surrounding the rust pustule, where chlorosis is the normal appearance. In severe cases the browning is marked and sporulation in the pustules is greatly reduced. High atmospheric humidity hastens and accentuates the browning at high temperatures. This seemingly abortive sporulation is common in many collections of race 34 and in certain collections of races 11 and 15, and it may occur in other stem rust races. It is most severe on Kota, Ceres, Kubanka, Aeme, Arnautka, and Thatcher wheats, on Vernal emmer, and on einkorn. It rarely or never occurs on Marquis, Little Club, Mindum, or Spelmar wheats.

Effect of Soil Reaction on Soil Rot of Sweet Potatoes. JOHN D. HARTMAN AND R. W. SAMSON. Sweet-potato soil rot was reduced by the use of sulphur and increased by the use of lime on Princeton and Elk sands having initial reactions of around pH 6.3 to

pH 6.9, as measured by the relative numbers and weights of marketable potatoes with no lesions and with small, medium, or large lesions. Increasing the acidity to about pH 5.9 or pH 6 greatly reduced soil rot and decreased yield little or not at all. Soil rot was more prevalent at pH 7.5 or higher and yields were lower. Among replicated plots adjusted to a given pH level soil rot tended to be more severe on those plots, which previous to adjustment had had the highest pH. The above pH determinations were based on samples taken in the spring.

Association of Bacterium Phaseoli and the Virus of Common Bean Mosaic.

FLORENCE HEDGES. *Bacterium phaseoli* has been isolated from bean plants inoculated with bean-mosaic virus, but showing a high percentage of bacterial infection on the inoculated primary leaves. Isolations were made from such primaries and also from trifoliates showing virus infection but no bacterial lesions. Cultures of the bacteria from each source produced 75 per cent typical mosaic and also bacterial infection on healthy bean seedlings 11 days and 6 weeks, respectively, after isolation. The bean-mosaic virus is ordinarily extremely "short-lived" outside the plant. In inoculations, both with a mixture of virus and bacterial cultures and in two parallel sets of serial passages of infected juice therefrom, begun September, 1936, and still in progress, dominant virus symptoms and rather inconspicuous bacterial ones with an occasional passage showing severe bacterial infection appeared to be the rule. In the serial passages, however, after 25 months, the bacterial symptoms suddenly disappeared for 10 successive passages. Shortly after this disappearance, there arose with the 38th serial passage (both sets) an ultra-severe mosaic still persisting (50th serial passage). Bacterial symptoms reappeared. Dissociation experiments are in progress with *Bact. phaseoli* from Lima-pod and 2 highly virulent isolations after 2 years of serial passages. Comparative studies are being made of "atypical" forms arising therein and those appearing in the association studies.

Spraying Tomatoes for Disease Control. R. G. HENDERSON AND S. A. WINGARD.

During the season of 1939, 22 varieties of tomatoes were sprayed with 3 spray preparations—yellow copper oxide (54Y), red copper oxide with cottonseed-oil emulsion, and a commercial preparation of red copper oxide combined with an emulsified oil (Fugroicide). Septoria leaf spot, Alternaria blight, and Phytophthora late blight occurred generally on all the varieties and were especially severe on the nonsprayed plants. The average yields per acre of all 22 varieties on the sprayed and nonsprayed plots were: yellow copper oxide, 15.4 tons; Fugroicide, 8.6 tons; red copper oxide and oil emulsion, 9.6 tons; nonsprayed check, 4.8 tons. Some varieties responded better to the sprays than did others. For example, yellow copper oxide on N. Y. State gave a 500 per cent increase in yield over that of the check plot, while, on Illinois Pride, the increase was only 69 per cent. The period of bearing was lengthened very greatly by spraying. Pritchard, normally an early variety, continued bearing throughout the season when sprayed with yellow copper oxide.

Maintaining Quality of Tomatoes by Delayed Spraying. JOHN W. HEUBERGER AND

JAMES G. HORSFALL. Ten years of field research on fruit quality are summarized. When *Alternaria solani* and/or *Septoria lycopersici* attacked, sprays reduced defoliation resulting in deeper red color and less sunscald and growth cracks. Spraying made fruits markedly firmer in 1938. Puncture readings by R. F. Suit in 1939 showed that spraying increased firmness by 11 per cent. Spraying reduced blossom-end rot in 1934, 1938, and 1939. Spraying increased percentage of U. S. No. 1 fruits in 1939 from 51.4 for nonsprayed to 66.0 for Bordeaux, and 70.6 for yellow cuprous oxide. "Insoluble" copper materials, in general, gave no spray injury. Delaying the applications reduced it for Bordeaux. In 1938 and 1939, respectively, stem-end-rot lesions (*A. solani*) were reduced from 34.1 and 7.2 for nonsprayed fruits to 19.6 and 4.5 for Bordeaux, to 21.3 and 4.7 for red copper oxide, and to 26.1 and 5.6 for copper oxychloride "A." Percentage anthracnose (*C. phomoides*) was reduced in 1938 from 29.2 for nonsprayed to 15.9 for red copper oxide and 12.3 for Bordeaux, and in 1939 from 7.5 for nonsprayed to 1.8 for yellow copper oxide, and 0.9 for Bordeaux. Delayed spraying effectively and inexpensively maintained fruit quality at a high level, despite disease.

Strains of the Fire-blight Organism. E. M. HILDEBRAND.

The cause of sporadic character of fire blight and the fluctuations in severity of its attacks has been sought in the pathogen. This study employed 136 isolates from diverse sources, involved numerous morphological, physiological and pathogenetical experiments and required 5 years for its completion in 1937. The most satisfactory susceptible material for measuring grades of virulence was slices of green Kieffer pear fruits. The effect on virulence of successive bi-weekly transfer to nutrient broth demonstrated more variability than stability during 5 years. The smallest organisms were commonly most virulent. The mean length meas-

urements of 63 cultures (1.59 μ), when classified according to virulence, were: 9 high, 1.42 μ ; 12 medium, 1.57 μ ; 18 slight, 1.61 μ ; 18 nonvirulent, 1.59 μ . Differences in frequency of cells possessing flagella, number of flagella per cell, and length of flagella seemed not to be significant, except for slightly virulent or nonvirulent cultures. Physiological experiments employing 41 carbon and 18 nitrogen compounds and numerous miscellaneous cultural and other tests, failed to locate a more reliable criterion for evaluating strains of the organism than pathogenicity. While numerous small differences obtain between individual strains or isolates, no strain is believed of significance in any breeding program for disease resistance.

Yellow-red or "X" Disease: A New Threat to Peach Industry. E. M. HILDEBRAND AND D. H. PALMITER. Originally discovered in Connecticut in 1933, the Yellow-red or "X" disease is now found nearly across the Continent. All varieties of peach seem to be susceptible, but only the chokecherry (*Prunus virginiana*) among wild plants. Leaf symptoms appear on peach in mid-June and are continuously present thereafter till autumn. The sequence—leaf yellowing, irregular red spots, shot-hole, defoliation—is repeated year after year, but rarely results in death to orchard trees. However, in the greenhouse, trees die when completely defoliated. Fruit drop accompanies leaf fall, but small mummies often remain attached. On the chokecherry, the sequence—beautiful red and yellow foliage, coloring and rosetting and finally rosetting and death—usually appears in successive years, although with considerable overlapping. Maximum injury encountered in peach orchards during the first and second seasons was 62 and 96 per cent of trees infected. The extent of symptoms on individual trees ranged from single shoots to entire trees. Following inoculations by budding, symptoms may be delayed a year or longer. Eradication of the chokecherry, on which the disease spreads very rapidly, from the neighborhood of peach orchards is the best control measure. Chemicals offer most promise in preliminary experiments.

The Response to Certain Vitamins of Fourteen Species or Strains of the Myriangiales. ARTHUR B. HILLEGAS, FREDERICK KAVANAUGH AND ANNA E. JENKINS. The variations in the response of 14 species or strains of *Elsinoë*, *Myriangium*, and *Sphaeloma* to different vitamins have been observed. The cultures were grown in a medium of mineral salts, asparagine, and dextrose, and the same medium to which different vitamins had been added. Eleven species or strains grew for 3 subcultures in the absence of thiamin. The 3 remaining species required some growth substances other than the water-soluble vitamins of known composition.

Succession of Soil-inhabiting Fungi Attacking the Roots of Maize. WEN-CHUN HO AND I. E. MELHUS. About 11,000 isolates have been made from roots of maize collected in various parts of Iowa since 1935. The fungi isolated may be divided into 3 groups based on disease-inducing capacity. (1) Severe: *Pythium graminicola*, *Rhizoctonia solani*; (2) moderate: *Diplodia zeae*, *Gibberella saubinetii*, *Pythium debaryanum*, *Helminthosporium sativum*, *Penicillium oxalicum*; (3) slight to none: *Trichoderma lignorum*, *Aspergillus niger*, *A. flavus*, *A. tamarii*, *Fusarium moniliforme*, *Basisporium gallarum*, *Phytophthora cactorum*, *Monotospora* sp., and *Fusarium* spp. The succession of these fungi on maize roots is influenced by soil and environmental conditions. In most cases *Pythium debaryanum* became parasitic before and following emergence causing seed decay and stunting. Later, *Pythium graminicola* appeared on seedling root tips, inducing rapid necrosis. Concurrently, *Rhizoctonia solani* often appeared on the mesocotyls, eventually causing decortication of the roots. Subsequently, such organisms as *Gibberella saubinetii*, *Helminthosporium sativum*, or *Penicillium oxalicum* produced further necrotic lesions on the mesocotyl and basal part of the roots. Still later, *Fusarium moniliforme*, *Fusarium* spp., *Gibberella saubinetii* (certain strains), *Trichoderma lignorum* and *Monotospora* sp. were observed in root lesions. On host maturity, *Diplodia zeae*, *Fusarium* spp., etc., may develop extensively in roots and crown, leaving no evidence of the earlier prevalent organisms.

A Comparison of Pathogenic Races of Fusarium bilbigenum var. niveum. DONALD E. HOFFMASTER. Wilt of watermelon, caused by *Fusarium bilbigenum* var. *niveum*, is prevalent in parts of West Virginia. A wilt of muskmelon in the same locality often is attributed to this fungus, but a survey has not revealed a *Fusarium* pathogenic to muskmelon. Inoculations with many different isolates of the watermelon pathogen have consistently failed to produce wilt on muskmelon. Cultures of the muskmelon wilt pathogen obtained from Minnesota have failed to wilt watermelon, while readily causing wilt of muskmelon. The two pathogenic races are culturally and morphologically alike. Sectors obtained from the two pathogenic races of the fungus showed wide variation in virulence but no change in host specificity. Sectors of several other wilt-producing *Fusaria* showed

similar variations in virulence, but no change in host preference. Pathogenic *Fusaria* probably originated from nonpathogenic soil *Fusaria* by dissociation; but no evidence was obtained to support the theory that the muskmelon pathogen originated from the watermelon pathogen in this manner.

Relation of Color to Fungicidal Value of Insoluble Copper Compounds. JAMES G. HORSFALL AND JOHN W. HEUBERGER. Previously, it was reported (Phytopath. 29: 303) that fungicidal value (i.e. spore-killing capacity) of cuprous oxide increased as the wave length of reflected light shortened. Two years' laboratory tests at Geneva, New York, showed that a similar color relationship occurred among the so-called insoluble basic cupric fungicide powders, both pure and commercial. As the wave length decreased from 5100 Å (green) for basic sulphate and one oxychloride through 5000 Å (greenish blue) for silicate, 4900 Å (blue green) for zeolite, 4800 Å blue for another oxychloride, to 4700 Å (bluish purple) for oxobordo, the fungicidal value (no data on field performance) increased. It seems noteworthy that, irrespective of anion, a single curve fitted all points for fungicidal value and wave length. Although fillers complicate the procedure, in the few cases where sound data could be obtained, it appeared that the fungicidal value relationship is one of particle size, as in cuprous oxide, i.e., the shorter the wave length the smaller the particle. It seems significant that the cuprous oxide curve did not coincide with that for the cupric materials. This indicates that the cuprous oxides were more fungicidal than would be indicated by their wave lengths.

Bleeding Canker of Maple. F. L. HOWARD AND N. CAROSELLI. This disease is prevalent in several species of maple in home grounds, parkways, and nurseries in Rhode Island. Repeated subjection to Koch's postulates has proved the pathogen to be *Phytophthora cactorum* (identified by C. M. Tucker). The characteristic symptom is a reddish ooze from small fissures in cankers of the trunk and the scaffold branches. Infected inner bark, cambium, and sapwood develop a reddish brown necrotic lesion, which often exhibits an olive-green margin. The fungus produces a toxic substance, which causes wilting of leaves and dieback of branches as secondary symptoms. Since the systemic nature of the infection indicates possible control by internal medication, a simple injection technique has been developed. A di-nitro-cresol compound has given promising results in laboratory and field trials, since it does not injure the healthy tissues of the tree and acts as an antitoxin and a pathogen-inhibiting agent.

Peach-mosaic Virus Strain Studies. LEE M. HUTCHINS AND L. C. COCHRAN. In commercial J. H. Hale peach orchards in southern California, in which all of the trees are affected with peach mosaic, some trees, known to have been affected for 5 years, have not developed the severe symptoms expected on the J. H. Hale variety. Orchard surveys tend to show a shock effect after initial infection, followed by partial recovery, but not sufficient to account for individual tree differences. When mildly affected trees are budded with buds from severely affected neighbor trees, they develop severe mosaic symptoms; severely affected trees, conversely treated, remain severely affected. Groups of 10 J. H. Hale peach nursery trees were budded from mosaic-affected orchard peach trees showing, respectively, mild, medium, and severe symptoms. Within each group, all inoculated trees developed almost identical symptoms, and the latter corresponded in most cases with the degree of severity of symptoms in the orchard-tree source. Certain severely mosaic-affected orchard peach trees produced apparently normal sportlike shoots. Among several shoots thus observed, some have later become severely affected; others, after 3 years, still appear normal. Buds from the nonmottled shoots, grafted in peach nursery trees, transmitted mild mosaic, whereas those from severely mottled shoots transmitted severe mosaic. Strains of the peach-mosaic virus appear to be demonstrated.

A Bulb Disease of Lilies Caused by Fusarium spp. E. P. IMLE. A heretofore unreported bulb rot of certain species in the genus *Lilium* particularly *L. candidum* and *L. testaceum*, has been under observation and study for the past 3 summers. Isolations from more than 100 different bulbs from widely separated parts of the United States and from Canada, Holland, and France have consistently yielded strains of *Fusarium*. Some cases of imported French candidum lilies showed from 20 to 36 per cent diseased bulbs in September, 1938. Imported stocks examined in 1939 revealed only 4 per cent diseased bulbs. Inoculation of wounded seedlings, bulblets, and larger bulbs have reproduced the disease symptoms but some evidence indicates that wounds are not necessary for infection. Symptoms may develop after the bulbs are in storage or transit. The fungus usually is confined to the base of the scales, severing them from the basal-plate portion of the bulb. Plant symptoms consist of a yellowing or purpling and premature dying of the basal leaves. Flowering stems, when produced at all, are stunted and inferior. Diseased bulbs, when not destroyed outright, tend to split up and become smaller. Partial control by disinfection of diseased dormant bulbs has been obtained by a 30 min. treatment with 1:50 formaldehyde.

Elsinoë and Sphaceloma Species in the United States, Puerto Rico, and Guam. ANNA E. JENKINS AND A. A. BITANCOURT. A new species of *Elsinoë* on the rubiaceae host, *Randia*, from Puerto Rico, is described and the discovery and identification of *Sphaceloma batatas* on *Ipomoea batatas* from Guam (1937) is reported. Including these 2 species and *S. lippae* and *Sphaceloma* on *Plantago rugelii* recently discovered in Indiana, 18 described species of *Elsinoë* or *Sphaceloma* are now known in the United States, Puerto Rico, and Guam. Their host plants represent 17 families ranging from the Juglandaceae to the Compositae. The number of *Elsinoë* or *Sphaceloma* species known in continental United States has more than quadrupled in the last 10 years, whereas the world list has increased at least tenfold, the species now numbering as many as a hundred. The largest number of new species from any one country has been discovered in Brazil. Of the 18 species now known to occur in the United States, Puerto Rico, and Guam, practically half had become established as early as 1869-1893, and 3 and possibly 4 between 1911 and 1926. Five others are without record before 1930. Representative specimens constitute Fascicle II of "Myriangiales Selecti Exsiccati," of which Fascicle I represents the species known in South America up to January 1936.

Bacterial Leaf Diseases in Tobacco Beds in Relation to Field Infection. E. M. JOHNSON AND W. D. VALLEAU. In a 1937-1939 survey in western Kentucky, of 533 beds receiving Bordeaux twice, only 2 had wildfire and 2 angular leaf spot. Infections appeared in these beds 3 days after treatment and probably were present when treated. In this area wildfire was present in 278 and angular leaf spot in 266 of 777 nontreated beds. Tobacco, set from infected beds, develops infection soon after setting and the diseases become destructive sooner than in fields from disease-free beds. In 1939, 47 fields set from beds, 29 of which had wildfire, 13 of which had both wildfire and angular leaf spot, and 13 of which had angular leaf spot, developed 2 to 6 weeks after setting, 1 to 100 per cent wildfire infection and 1 to 76 per cent angular-leaf-spot infection. Despite low rainfall in the last half of the growing season, the damage in fields from these beds was 1 to 50 per cent. No damage occurred in 100 fields set from Bordeaux-treated beds, and infection was rare. Bordeaux gave similar results in 1936-1937. In 1938, a rainy season, Bordeaux delayed injury until a few days before cutting time, and some satisfactory crops were harvested.

A Buff Smut of Fall Panicum. H. W. JOHNSON, H. A. RODENHISER AND C. L. LEFEBVRE. In an experiment made at Arlington, Virginia, in 1938, to determine the effect of incubation period temperature on the pathogenicity of *Sorosporium syntherismae* on *Panicum dichotomiflorum*, 70.9, 72.5, 53.3, and 22.2 per cent smutted plants developed following 26 days' incubation of chlamydospore-dusted seed at temperatures of 5, 10, 15, and 20° C, respectively. One smutted plant in this experiment was observed to have buff sori composed of hyaline, smooth-wall chlamydospores, while the other 307 smutted plants all had the common black sori composed of brown, echinulate-wall chlamydospores. Inoculations made in 1939 with chlamydospores of the buff-type smut resulted in 68 smutted plants, all of which produced only buff sori. Parallel inoculations with chlamydospores of the dark-type smut resulted in 240 smutted plants, all of which produced only black sori. Single chlamydospore cultures of the two types of smut were established and the optimum temperature for the growth of each type on potato-dextrose agar was approximately 20° C. The cultures of the buff smut were mycelial and made greater radial growth than did cultures of the black smut, which were of the sporidial type. It appears that the buff smut is a result of mutation in *Sorosporium syntherismae* and that the change may involve several genetic factors.

Inoculation of Bean with Extracts from Other Healthy Legume Species. JAMES JOHNSON. Juice extracted from 122 apparently healthy species (representing 50 genera) of the Leguminosae was rubbed with carborundum into leaves of young healthy beans (*Phaseolus vulgaris* L. var. Stringless Green Refugee). No species has consistently yielded a virus on the host plants used. Juice from single plants of one species, *Lathyrus tingitanus*, has been applied as inoculum more than 100 times to several bean plants and has induced systemic symptoms in every instance. Serial transmission, however, failed; the properties of the agent are not characteristic of a virus. The response of the bean to the extract from the Tangier pea (*L. tingitanus*) is tentatively interpreted as an allergic reaction. Two seemingly new viruses have been obtained from inoculations with apparently healthy plants. One of these has been obtained several times and the other only once in several attempts. It is logical to assume that these viruses were seed-borne and that the host species are symptomless carriers. If some part of the protoplasm of one species should, however, find favorable conditions for growth in the cells of another, when properly introduced (viroplasm theory), it is believed that consistent results should not, necessarily, be expected from such transfers.

A Rare Virus Disease on Tobacco. JAMES JOHNSON AND ROBERT W. FULTON. In 1938 a 6-acre field of tobacco near Edgerton, Wisconsin, showed about 30 per cent of the plants infected with an apparently new ring-spot virus. Similar symptoms had not been seen in extensive field disease surveys in previous years, nor were they noted in other fields in 1938, and did not appear in the original field in 1939. Of the several ring-spot viruses described, the new virus resembles most closely tomato ring spot, as described by Imle and Samson. Because the chlorotic rings are usually broader than those of other known ring-spots, the virus is tentatively given the common name "tobacco broad ring spot." The virus has been transmitted to some species outside the Solanaceae, including cucumber and sunflower. It apparently is not transmitted by *Myzus persicae*. The thermal death point after 10 minutes exposure is 52° C., the dilution end point is about 1 in 750, and the tolerance to aging *in vitro*, 36-48 hours at room temperature. Protective inoculation trials with ordinary tobacco ring spot showed no immunological reactions. Such rare occurrences of virus infection is of some interest in relation to the nature and origin of virus diseases.

A Bud-transmissible Chlorosis of Prunus cerasus. G. W. KEITT AND C. N. CLAYTON. A chlorotic disease of sour cherry, previously called "physiological yellow leaf," has been transmitted by budding. A typically diseased tree shows chlorosis and abscission of the older leaves, usually beginning about 3 weeks after petal-fall. Length of twig growth does not seem markedly affected, but the spur system becomes progressively reduced. The trees become unprofitable, producing sparse crops of large fruits free from necrosis or bumpiness. In 1938, diseased buds were inserted in 24 healthy Montmorency trees in 3 orchards. Twenty trees showed typical chlorosis in 1939; 4 were doubtful. Only 3 of the inserted buds produced shoots. Healthy buds were inserted in 8 diseased trees in 3 orchards. Four grew into shoots, all of which showed the characteristic chlorotic leaves and defoliation. Fourteen control trees, in one of which healthy buds were inserted, showed no symptoms of the disease. Microscopic and cultural examination of diseased tissues revealed no causal fungus or bacterium. Orchard records for 4 years show a steady increase in incidence of the disease. The evidence indicates the disease is caused by a virus. Its further investigation and inquiry into its possible relations to previously described viral diseases are in progress.

Eradicant Fungicidal Treatments in Relation to Apple-Scab Control. G. W. KEITT, C. N. CLAYTON, AND M. H. LANGFORD. A small Wealthy orchard at Madison, Wisconsin, was sprayed in October, 1938, with a copper-lime-arsenic mixture. A similar orchard nearby was not treated. Neither was sprayed in 1939. Counts indicated reduction in perithecia per unit area of overwintered leaf surface in the treated orchard, as compared with the nontreated, of 98 per cent; of current-season lesions per leaf, May 29, 99 per cent; June 26, 96: of lesions per fruit, Sept. 1, 98. Sixty-six per cent of the fruit of the nontreated orchard was scabbed, 4 per cent of the treated. The floor of a 6-acre McIntosh orchard at Sturgeon Bay was sprayed before bud-break with 1 per cent "Elgetol Extra," 450 gal. per acre. A small orchard 0.3 mile away served as control. Counts indicated that, through the critical period until 3 weeks after petal-fall, the Elgetol treatment reduced by about 0.9 the unusually severe epidemic. Seven lime-sulphur treatments in the orchard that received Elgetol and 8 in a similar one that did not gave, respectively, 1 and 32 per cent scabbed fruit at harvest. Reduced summer-spray programs were more effective in the orchard that received Elgetol than in others that did not.

Variation in the Germination of Chlamydospores of Ustilago zeae. M. F. KERN-KAMP AND M. A. PETTY. Germination of chlamydospores of *Ustilago zeae* was studied on agar drops on cover slips on van Tieghem cells, the environment being held constant. Extreme variations from the classical 4-cell promycelium were observed in the 14 crosses and collections studied. Likewise, there were variations in the number of promycelial cells giving rise to hyphal branches or sporidia, the number of chlamydospores from which sporidia grew directly, the number of chlamydospores having promycelial cells on 2 sides, and the number of promycelial cells producing neither sporidia nor hyphal branches. The germination type varies with the cross or collection of smut. For example, the number of 4-cell promycelia varied from 11 per cent to 78 per cent in different crosses and collections. The differences between crosses and collections were much greater than differences within crosses and collections, and one type of germination usually predominated in each cross or collection. As the environment in which the spores were germinated was held constant, differences probably are due to genetic differences between the crosses and collections.

Physiologic Race Determination in Puccinia coronata avenae. C. H. KINGSOLVER AND H. C. MURPHY. A study was made of the reaction of 21 oat varieties to 81 single-pustule isolates of *Puccinia coronata avenae* collected in 16 states in 1938. These varieties

were those used for physiologic-race determination by Murphy in the United States and by Straib in Germany. Using Murphy's 13 varieties 19 races could be identified; with Straib's 15 varieties 46 races; with the 21 varieties 54 races. Twenty-four isolates identified as race 1 (Murphy) could be divided into 18 races by their reaction on Straib's varieties. Similarly, 28 isolates identified as race 6 (Murphy) could be divided into 16 races. Twelve isolates giving the same reaction on Straib's varieties could be divided into two races by reaction on Murphy's varieties and these were races 1 (3 isolates) and 6 (9 isolates). Of the 19 races identified with Murphy's varieties, 13 had been previously described and 6 were new. Races 1 and 6 in almost equal proportions comprised over 50 per cent of the total number. Race 47, first identified by Waterhouse in Australia, was identified in 2 collections—its first report in the United States. Race 7, one of the most prevalent races in the past, was identified only twice. Cooperative investigation between U. S. Department of Agriculture and Iowa Agricultural Experiment Station.

Diseases of Citrus. L. J. KLOTZ. A hundred color photographs and brief descriptions of nutritional, virus, mechanical, and parasitic disorders.

Studies on Phytophthora citrophthora. L. J. KLOTZ. Optimum growth temperature is 26° C. Mature zoösporangia are produced in 4 to 5 hours on alfalfa-stem cultures in running, well-aerated water at 24° C. In both light and darkness, decrease in temperature from any point between 10 and 29° C. induced swarming; an increase did not. At 9, 15.5, and 19° C. many zoöspores (5 to 20 per low-power field) were motile after 10 hours; but none after 24 hours; at 12.5° C. many (5 to 10) were motile after 24 hours; below 7° motility was lost in a few seconds and at 24° C., usually within an hour. At 25° C. germ tubes developed to an average length of 450 μ after 24 hours in nonsterilized tap water, 550 μ in distilled water, and 816 μ in 1-to-1 citron albedo sap; they penetrated lemon rind to a depth of 600 μ in the same period. One minute at 44.4° C., two at 43.9° C., and five at 43.3° C. were lethal to both zoöspores and mycelium. They survived 9 days' continuous freezing at -6.5° C., but were killed by 1 day at -20° C. and 1 day at -20.6° C. The fungus on alfalfa stems in hardware cloth buried in the field was not viable after 24 hours at the 1-inch depth, the temperature attaining 36.4° C. in shade and 43.9° C. in sun. After 12 days it was not recovered from the 2-inch level, but was cultured from the 3- and 6-inch depths, temperatures reaching 44.4° C., 40.0° C., and 32.2° C., respectively, at the 3 levels. In autoclaved soil, with 5 or 10 per cent moisture, it did not survive 20 hours at 40° C. or 70 hours at 35° C., but was recovered after 43 hours at 35° C. One part cuprion in 300,000 prevented germination; $\frac{1}{4}$ - $\frac{1}{8}$ -100 Bordeaux protected lemon fruits.

Rapid Seed-corn Drying Checks Seed Infection. BENJAMIN KOEHLER. Corn ears of several widely grown hybrids were hand-picked in the field in 1937 and 1938, when the grain moisture was about 30 per cent. The ears of each hybrid were divided at random into 3 lots of 120 ears each to determine the effect of different rates of drying on internal seed infection. Chambers with temperature and humidity control were used in which the ear corn was dried to 12 per cent grain moisture. One lot was dried at 106° F., 32 per cent relative humidity, for 4 days; another lot at 70° F., 65 per cent relative humidity for 4 weeks; and the third lot at 70° F., and 86 per cent relative humidity for 3 months. Rapid drying avoided internal seed infection to a marked extent in these tests with selected ears of sound appearance. Total internal seed infection with the various fungi concerned was 5.1, 18.3, and 69.0 per cent, respectively, for the 3 different drying conditions. *Fusarium moniliforme*, *Gibberella zeae*, *Nigrospora* spp. and *Penicillium* spp. were all markedly increased by retarded drying. Rapid drying, as now practiced by many hybrid seed corn producers, seems of some value in controlling disease.

Seed Treatment for the Control of Bacterial Bean Blight. K. W. KREITLOW. Seed treatments for control of bacterial bean blight were tested in laboratory and field. Laboratory tests of diseased seed have given information on length of time seed can be treated without injury, as well as effectiveness of treatments in killing the disease organisms. On the basis of this information, 4 disinfectants have been used in treating several different lots of diseased seed prior to planting in the field. Six separate treatments and plantings were carried out at weekly intervals in the spring of 1939. The total average blight percentage in all checks for all plantings was 23.6 per cent, whereas the total average blight percentage of the 4 treatments for all plantings was less than 0.20. Yields of from 2 to 7 times that of the checks were obtained, and the quality of the beans was correspondingly higher in the treated plots. Periodic germination tests on treated bean seed kept in storage revealed no important decrease in germination during 7 months. The following solutions were effective in controlling bean-blight bacteria without serious injury due to the seed coats slipping: (1) 1:500 mercury bichloride in di-ethyl ether. (2) 1:20,000 brilliant green in 50 per cent ethyl alcohol plus 3 per cent

acetic acid. (3) 1:500 mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid. (4) 1:20,000 gentian violet in 50 per cent ethyl alcohol plus 3 per cent acetic acid.

A Necrotic Virosis of Cabbage. R. H. LARSON AND J. C. WALKER. A virosis of cabbage associated with but distinct from mosaic has been observed at Madison, Wisconsin. The symptoms resemble those of black ring in the paucity of mottle and absence of vein clearing, together with the preponderance of systemic appearance of small necrotic rings or spots. Black or purple, irregular, necrotic, slightly sunken lesions occur on stems. This virosis is distinct from black ring in that it develops best at 22 to 25 degrees C. and is completely masked at 13° to 19°. The physical properties also differ from those of black ring and mosaic. The inactivation point is 50°, duration *in vitro* 24 hours at 22°, and the dilution tolerance 1 to 500. It is transmitted readily by mechanical means and by the cabbage and peach aphids. It affects systemically all crucifers tested and also beet, chard, spinach, cucumber, zinnia, calendula, petunia, *Nicotiana glutinosa*, *N. rustica*, *N. langsdorffii* and *N. repanda*. Local lesions only are produced on inoculated leaves of tobacco. (University of Wisconsin and Division Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.)

A New Phoma Disease of Perennial Delphinium. THOMAS LASKARIS. A hitherto unrecognized disease caused by a species of *Phoma* has been frequently found in plantings of Delphinium in New York, New Jersey, and Connecticut. In the field the disease produces cankers, usually near the crown, causing one or more cracks in the stem, which may extend upward for a considerable distance. Pycnidia of the fungus can be found in the blackened tissue adjacent to the edges of these cracks and also on irregular black or brown necrotic areas along the stem. Another characteristic symptom is the blackening of tissue at the base of each leaf petiole on which areas pycnidia develop in abundance. On old stems reddish-brown masses of pycnidia may appear near the top. The fungus has been isolated from the areas mentioned above and from crowns and roots of other naturally infected mature plants. Recently the fungus has been isolated from reddish-brown areas on leaves and petioles of young seedlings in the greenhouse. Inoculations on greenhouse plants have been successful and there is no doubt as to the pathogenicity of the fungus. The importance of this organism as a cause of crown rot of perennial delphinium is being investigated.

Fungi Associated with Scolytus multistriatus in Regions where Ceratostomella ulmi Has not Been Found. J. G. LEACH. A survey has been made of the fungi associated with the smaller European elm-bark beetle in southwestern West Virginia where the Dutch elm disease has not been found. The following fungi appear consistently associated with the insect, and they fruit abundantly in the brood galleries in a manner adapted to effective dissemination by emerging beetles: 1. a species of *Graphium* that forms coremia in the tunnels in a manner closely paralleling that of *Ceratostomella ulmi*; 2. a white *Penicillium* that sporulates in the egg galleries during larval development; 3. a species of *Fusarium* that forms microconidia in Cephalosporium-like heads; 4. one or more species of yeast. These fungi are believed to play an important rôle in the economy of the insect by creating a microenvironment favorable for larval development. The beetle is multiplying rapidly in West Virginia in trees killed by phloem necrosis, and conditions exist that will be conducive to rapid spread of the Dutch elm disease if and when *Ceratostomella ulmi* gains access to the region.

The Reniform Nematode as a Root Parasite. M. B. LINFORD, JULIETTE M. OLIVEIRA AND FRANCIS YAP. A nematode (description in press) that necessitates erection of a new genus of the Tylenchidae occurs on Oahu, Territory of Hawaii, as a mildly pathogenic parasite of roots. Over 60 known host species represent 29 families of plants. Injuries include minute cortical lesions and mild hypertrophy in the stele. Larvae of typical eelworm shape develop in the soil, without feeding, into mature nonfeeding males and infective young females, both resembling larvae in size and shape. Females penetrate intracellularly into the cortex of roots, coming to rest with the feeding stylet inserted into an endodermal cell. This cell and nearby pericycle parenchyma enlarge and become densely protoplasmic. The posterior part of the female, usually on the root surface, enlarges to become reniform, then eggs are laid in a gelatinous matrix, forming a mass that covers the reniform body and adheres to root and soil. In naturally infested soil, of a moisture content near the wilting percentage for plants, the reniform nematodes exceeded 50 per cent survival during 36 weeks. In soil air-dried 33 weeks, with moisture dropping to $\frac{1}{2}$ of the wilting percentage, there was more than 5 per cent survival. This nematode appears less tolerant to freezing in soil than does the root-knot nematode.

Hyperauxing of Nodules of Red Kidney Bean, Soybean, and Garden Pea. G. K. K. LINK AND VIRGINIA EGGERS. Using the *Avena* coleoptile test and peroxide-free ether ex-

tracts of the test material, it was shown that the auxin content of nodules (1) of kidney bean is 3-10 times greater; (2) of soybean is 1.5 times greater; and (3) of garden pea is 2.5 times greater, respectively, than that of the roots that bore them. The auxin contents of nodule-free root systems of these plants, when grown in sterile quartz with Shive's complete nutrient solution, were approximately the same as those of nodule-bearing plants grown in garden soil.

Dissemination of the New York Aster-yellows Virus and Its Leaf-hopper Vector, Macrosteles divisus in Endive Beds. M. B. LINN. A study of the dissemination of the New York aster-, lettuce-, and endive-yellows virus has shown that the amount of yellows in certain endive beds is often considerable in plant rows immediately adjacent to bordering weeds, gradually decreasing in amount towards the far sides or ends of the beds. Yellows-distribution curves, having as coordinates percentage of infection and distance in feet from sampling point to weed borders, are typically logarithmic. If the logarithm of the percentage of infection be plotted against the distance in feet, the resulting curves are straight lines. This suggests that vector and virus dispersion, as measured by the incidence of yellows, may be expressed by constants that vary in value from one bed to another, and are conditioned by any factors influencing feeding and dispersion of the vector. Knowing the intercepts or points where the log curves intercept the coordinates, one may then postulate the highest percentage of infection that could be anticipated in the bordering weeds, and the distance in feet from the weeds to that point in the endive beds, where the former were no longer factors in the distribution of yellows in these beds.

The Epidemiology and Control of Hop Downy Mildew. ROBERT O. MAGIE. Under New York conditions, oospores of *Pseudoperonospora humuli* in the soil are the principal means of overwintering. Sporangia on detached leaves withstood drying for 27 days over calcium chloride or in the greenhouse at summer temperatures. Plants were sprayed with sporangial suspensions, placed in moist chambers at 10, 13, 18 and 24° C., then removed, and quickly dried after various time periods. Minimal period of wetting permitting infection at the two higher temperatures was 1½ hours and maximal infection occurred at 13° and 18° after 24-hour wetting periods. Removal of diseased shoots or application of calcium cyanamid to crowns retarded secondary infection, but did not permit a reduction in spraying. Bordeaux and cuprous oxide were more effective than other fungicides tested. Bordeaux injured young leaves and stunted cones. The addition of ½ per cent cottonseed oil reduced these injuries. Bordeaux was outstanding in that rain drippings from sprayed leaves protected nonsprayed growth. Four biweekly applications, beginning the middle of June, of either Bordeaux 4-2-100 or cuprous oxide 1-100, with the oil, were very effective if thoroughly applied. It is suggested that spraying may be delayed until infection reaches the foliage 5 feet above ground.

A Comparison of Methods of Laboratory Spraying for the Testing of Protective Fungicides. S. E. A. MCCALLAN AND FRANK WILCOXON. The errors of testing fungicides in the laboratory arise from 2 major sources: biological, involving the fungus, and mechanical, arising from application of the spray. A comparison was made of 3 basic principles of applying sprays: freehand spraying, spray chambers, and settling towers. Uniformity and reproducibility of spray deposit were determined by spraying with a dye solution and measuring in a colorimeter. The coefficient of variation for spray deposits on replicate slides sprayed at (a) the same time, and (b) different times or days, was as follows: (1) freehand spraying with bulb atomizer, (a) 31.0 per cent, and (b) 98.7 per cent; (2) freehand spraying with controlled pressure and time - 8.0 and 63.0 per cent; (3) spray chamber, fixed nozzle, controlled pressure, concentration varied by time of spraying - 5.9 and 30.1 per cent; and (4) settling tower, controlled pressure and time, concentration varied by successive exposures - 4.5 and 12.8 per cent. Method (1) is entirely unsatisfactory. The chi-square test applied to toxicity curves for 2 fungicides on the spores of 4 different fungi gave the following mean chi-square/n values: method (2) 7.4, (3) 4.3, and (4) 2.6. A permanent stainless steel settling tower suitable for laboratory testing of sprays and dusts has been designed and constructed.

Field Studies on Paradichlorobenzene in the Control of Tobacco Downy Mildew. RUTH MCLEAN AND J. A. PINCKARD. Previous investigations have demonstrated the fungicidal value of paradichlorobenzene vapor to lie in its specific or eradicant action on the parasite without causing significant injury to the host. This important property of the fungicide allowed delayed seed-bed application of the vapor until infection had occurred and the sporangia of downy mildew appeared on seedlings. Following appearance of the parasite, in a given seed bed of 100 sq. yd., 3 to 4 lb. of No. 6 size crystals were broadcast on the ordinary cotton cover at 6 p.m. A cotton fumigation cover having 64 warp and 64 woof per inch was drawn over the treated seed bed, where it remained 12 hours. On farm-oper-

ated seed beds, 3 nightly treatments of 12 hours each sufficed to eradicate the parasite when temperature was above 6 to 8° C. Single 12-hour treatments may suffice on warm nights, using wet or sealed fumigation covers. Treatments made nightly and on alternate nights with half the above amount of paradichlorobenzene were effective. Damage to seedlings resulted from the vapor at temperatures encountered during attempted day treatments of shorter duration. Vapor concentrations in seed beds were determined quantitatively and correlated with fungicidal effectiveness.

Adaptive Parasitism of Phytophthora infestans. W. R. MILLS. The virulence of *Phytophthora infestans* has been increased by passage through a series of potato plants representing 3 different levels of resistance. Varieties entirely immune from the original cultures become completely susceptible to the virulent ones. Plants representing a fourth level of resistance are immune from all cultures. Increase of virulence occurs while the mycelium is growing within the tissue of the resistant plant but not when the fungus is propagated in susceptible hosts. Somatic fragments (swarmspores) of increased virulence are selected by applying them to foliage of greater and greater resistance. The virulence of the identical cultures used in the experiments with potato has been progressively increased for tomato by 7 successive passages through foliage of that host, at which time the fungus produced a blight of tomato fully as severe as that caused by a culture taken from a naturally infected potato. Increase of virulence on tomato did not affect in any manner the virulence for potato. The converse also proved to be true. Once increased, virulence of cultures could not be reduced by long periods of growth on tubers and foliage of low resistance.

Auxin Production by Ustilago zeae Grown on a Medium Free of Tryptophane. J. E. MOULTON AND G. K. K. LINK. *Ustilago zeae* was grown on Ranker's synthetic nutrient solution in which dextrose is the source of carbon and the only organic substance present. The organism was grown on this medium for 8 weeks. Ether extracts were made from the fungus mat and the supporting medium, using the extractor and methods employed by Link, Wilcox, and Eggers (Phytopathology 28: 15, 1938). The extracts were applied to *Avena* coleoptiles and were found high in auxins. These results indicate that *Ustilago zeae* is capable of producing auxins when grown in a synthetic medium devoid of peptone or other sources of tryptophane. Work is under way on the relationship of auxins and gall production by strains of *U. zeae*.

Effect of the 1938 Crown-rust Epiphytotic on Yield, Bushel Weight, and Lodging of Oats in Iowa. H. C. MURPHY, L. C. BURNETT AND C. H. KINGSOLVER. A statistical study was made of the effect of the severe 1938 crown-rust epiphytotic on the yield, bushel weight, and lodging of 442 varieties and selections of oats grown in replicated yield tests at Ames and Kanawha, Iowa. Coefficient of infection (a product of percentage and type of infection) was more highly negatively correlated with yield and bushel weight than either percentage or type of infection. The 442 selections, mostly of hybrid origin and varying in reaction to crown rust from near immunity to complete susceptibility, were grouped according to their coefficients of infection. Their average yields ranged from 24.9 bushels per acre for those with 91-100 coefficient to 79.7 for those with 0 rust. The correlation between yield and coefficient of crown-rust infection was -0.8. For each unit increase in coefficient of rust, yield was decreased 0.31 bushels per acre at Ames and 0.27 at Kanawha. Bushel weight showed a similar high negative correlation with rust infection. Lodging was increased by increase either in yield or crown-rust infection, or both; and, since yield and rust were negatively correlated, the effect of lodging (caused by rust) on yield tended to be nullified. (Cooperative investigation between U. S. Department of Agriculture and Iowa Agricultural Experiment Station.)

Progress in Onion-smut Control by Seed Treatment. A. G. NEWHALL. Although the liquid-formaldehyde-drip method of controlling onion smut on muckland is usually effective in reducing the disease to less than 5 per cent, weight of the solution adds much to the labor of sowing. In the past 5 years formaldehyde dusts, used in a similar manner, have proved neither so effective nor so economical in money or labor. More promising results have been secured recently by employing organic fungicidal dusts in seed treatment. Slow, gradual release of the fungicide is considered an important element in their success. The first to be tested, Brassicol or Brassisan (20 per cent pentachlor-nitro-benzene) was too toxic to onion seedlings, although it controlled smut fairly well. Two other organic compounds have given good smut control with sufficiently unimportant seed injury to warrant field trials on a larger scale. Mean percentages of smut on seedlings in 3 fields in 1939 were checks 32 per cent, liquid formaldehyde 3.9 per cent, DuBay dust 120U (50 per cent tetramethylthiuram disulfide) 2.49 per cent, DuBay dust 1242CC (50 per cent ferrie dimethyldithiocarbamate) 2.51 per cent. Dust needed per pound of seed (1: 1) requires a sticker, such as soluble cottonseed oil or rosin-lime-sulphur for best results.

Onion Bloat or Eelworm Rot Caused by the Nematode Ditylenchus dipsaci. A. G. NEWHALL AND B. G. CHITWOOD. A second outbreak of the bulb or stem nematode was discovered on the mucklands of New York State in the summer of 1939. The fact that this disease is not recorded for any other State is very likely due to failure to recognize it. General field symptoms may be taken for lightning injury, but their recurrence in the same location is an important difference. Seedling symptoms include a twisting of the cotyledon on emergence, a white discoloration, enlarged areas and later a broken epidermis on the first leaf. Symptoms on half-grown onions raised from sets include stunting, prostrate outer leaves, die-back symptoms above ground, and, late in the season, a softening of the outer scales. An increased number of splits and doubles often occurs, as well as many secondary rots. On a mature bulb the diagnostic symptom is a soft white mealiness of the inner surface of the outermost scale or two, in which location the nematode usually can be found in abundance. The pathogen also is often present in stem and leaf tissue of growing onions late in August. Infested bulbs are not characterized by excess moisture or disagreeable odor. Parenchyma continues to break down in storage. The puffy soft condition of the second scale has suggested the term "bloat."

Studies on the Fungicidal Properties of Silver. L. W. NIELSEN. Freshly precipitated silver arsenate, arsenite, carbonate, chloride, chromate, cyanide, dichromate, phosphate, nitrate, sulphate, colloidal silver, and 3 silver-soap mixtures were found to be strongly fungicidal to *Alternaria solani* when tested on excised potato leaves. Silver bromide, ferrocyanide, iodide, sulphide, thiocyanate, thiosulphate, and nucleinate were only weakly fungicidal. Under greenhouse conditions silver-lauryl sulphate and -oleyl sulphate precipitates and precipitated silver oxide with silver concentrations of 12.5 p.p.m. controlled late blight infection on potted potato plants, as well as 4-4-50 Bordeaux mixture. During the past summer silver-lauryl sulphate and -oleyl sulphate precipitates, silver dichromate, arsenate, cyanide, oxide, chloride, and colloidal silver were tested under field conditions. The protection given potatoes against late blight by silver chloride and silver-lauryl sulphate precipitate was inferior to that given by Bordeaux mixture, as was apple scab control with silver oxide, dichromate, and silver-lauryl sulphate precipitate. Laboratory studies have shown that the silver sprays tested adhere very poorly. Various organic and inorganic materials are being added to or combined with silver in an effort to increase its adhesiveness and still retain its toxicity. Sprays containing silver and sodium lauryl sulphate or sodium oleyl sulphate have injured in some cases.

The Character of Supplements and Their Effect on the Performance of Copper Fungicides. A. A. NIKITIN. An attempt was made to correlate the effect of supplements on the solubility and degree of adherence of copper fungicides. These studies were relative to the performance of copper fungicides when used in combination with supplements, both in the laboratory and in the field. The results secured on the study of the physical, chemical and toxic properties of copper fungicides definitely showed that the addition of supplements may greatly change the performance of copper fungicides. The prime function of supplements is to improve the physical properties of the copper fungicides, such as adherence and spreading. However, in performing this function, they should be chemically unreactive with the copper fungicides. It is essential that the supplements should be of such a character that they do not readily undergo oxidation, reduction or hydrolysis, on exposure to atmospheric action. In this respect, they should not change the solubility of the copper fungicide. It has been found that among the materials commonly available on the farm soya flour, wheat flour, casein, and corn oil make suitable supplements. Skimmed sweet milk improves the adherence and spreading properties of copper fungicides, and, in addition, insures a very good finishing of the fruit.

Eradicant Treatments as an Aid in the Control of Apple Scab. D. H. PALMITER. The value of eradicant treatments in reducing the primary inoculum of *Venturia inaequalis* was tested in 2 isolated McIntosh orchards on the same farm. One block of 55 trees received a thorough ground application of Elgetol ($\frac{1}{2}$ per cent). Most of the second orchard received an application of a nitrate-arsenite solution (NaNO_3 100 lb., CaAsO_2 4 lb., water 100 gallons), but one end was left untreated. These materials were applied at the green-tip stage with a spray gun and sufficient pressure to kick up the clumps of old leaves and force the chemicals to the lower layers. The ground was sprayed from 2 directions with a total of 500 gal. per acre. Scabbed fruit on nonsprayed trees of the 2 treated plots and the nontreated plot was 2.7, 3.4, and 72.0 per cent, respectively. Trees receiving only the calyx and 10-day sprays (floatation sulphur paste 6-100) had 2.0, 3.1 and 43.1 per cent infection, respectively; while those receiving 4 sprays had 0.2, 0.8 and 1.7 per cent infection, respectively. From these and previous years' data it is concluded that such eradicant treatments can reduce a potentially heavy ascospore inoculum to such low levels that mild fungicides may be used with comparative safety.

The Soil Rot, "Pox or Pit," of Sweet Potato. L. H. PERSON. Two types of symptoms have been recognized in Louisiana as an accompaniment of the disease or disease complex of sweet potatoes known as soil rot, pox, or pit. These are: (1) the symptoms first given by Halsted and characteristic of a widely distributed disease; (2) the symptoms of the very serious disease that has been spreading in Louisiana during the past 5 years. From potatoes affected with the latter, or the Louisiana disease, there has been isolated an *Actinomyces* capable of reproducing the disease. This organism is apparently new and will be described as *Actinomyces ipomoea*, n. sp. The place in the complex of those potatoes showing the symptoms first described by Halsted is not yet clear.

A Laboratory Method for Determining the Fungicidal Value of Vapors and its Application to Paradichlorobenzene in the Control of Tobacco Downy Mildew. J. A. PINCKARD AND RUTH McLEAN. Atmospheric concentrations of paradichlorobenzene vapor, fungicidal to downy mildew infected tobacco seedlings growing in jars, were studied by a simple semi-automatic, air-saturation method. Known concentrations of the fungicide, approximating a precision of 3 parts per 1,000, were delivered to test plants continuously or intermittently as required. The sporangial cycle of the parasite being 6 days, vapor treatments of infected seedlings were not begun until the 3rd day after inoculation. Harmless prevention of sporangial formation was correlated with vapor concentrations for single 12-hour treatments, and for alternate 12-hour treatments. With repeated alternate treatments, concentrations as low as saturation at 0° C. (0.01 volume per cent) were fungicidal. Single 12-hour treatments were fungicidal if concentrations equal to saturation above 7° C. (0.02 volume per cent) were applied. Concentrations at 13° C., or above, (0.04 volume per cent) were injurious to plants after 12 hours. Paradichlorobenzene vapor causes changes in parasite or host, or both, resulting in destruction, or prevention, of parasitism without causing significant harm to the host. To be fungicidal the vapor must be soluble in the cell plasma. Since these solutions obey the gas laws approximately, atmospheric concentrations are, at best, merely indicative of the important tissue concentrations.

Phytophthora Disease of Maples. P. P. PIRONE. During 1938 and 1939 hundreds of maples, especially *Acer platanoides*, have died throughout New Jersey. An early symptom of the disease is a thin crown resulting from a decrease in the number and size of the leaves. Trees die within a year or two following this period of weak vegetative growth. The presence of cankers at the base of the trunk near the soil line is the most striking symptom. The inner bark, cambium, and often the sapwood are reddish brown in the cankered area. Death occurs when the cankers completely girdle. Isolations from cankered and discolored areas yielded a fungus identified by C. M. Tucker, University of Missouri, as belonging to the *Phytophthora cambivora* group. Pathogenicity of this fungus has been established; it produced typical symptoms on at least 12 artificially inoculated trees; in all cases it was successfully reisolated. Infection resulted only when trees were wounded just before the inoculum was applied; no infection occurred when the fungus was applied to unwounded maple bark. Wound inoculations of young rhododendrons resulted in symptoms typical of the disease induced on it by *P. cambivora*. Application of the *Phytophthora* in wounds on *Cornus florida* did not produce infection.

Brown Scale Disease of Easter Lily. A. G. PLAKIDAS. During the last few years the brown-scale disease, a serious trouble of the Easter lily, also known locally as "brown bulb" or "black bulb," has become increasingly worse in the lily-growing district of Louisiana. The disease—apparently soil-borne—is characterized by a brown discoloration, mainly of the outer scales, although smaller brown spots may be scattered locally on the inner scales. When the bulbs are first dug, the brown areas are more or less superficial, the injury to the tissue rarely extending deeper than about $\frac{1}{4}$ to $\frac{1}{2}$ mm. Later, in storage, the affected scales shrivel, dry up, and turn dark brown to black and the bulbs become unmarketable. Repeated plantings from affected tissue yielded mainly 3 fungi, *Penicillium* sp. (probably *P. cyclopium*), *Fusarium* spp., and one which has been tentatively identified as *Vermicularia* sp. Typical symptoms have been produced on healthy bulbs planted in sterilized soil inoculated with the last named fungus, either alone or in combination with the *Penicillium* or the *Fusarium*, or both. As neither the *Fusarium* nor the *Penicillium*, alone or together, produced infection, the *Vermicularia* appears to be the cause of the brown-scale disease.

*Effect of Some Mineral Nutrients on Development of Clubroot (*Plasmodiophora brassicae*).* DEAN E. PRYOR. Several resistant and susceptible crucifer varieties were grown in sand artificially infested with *Plasmodiophora brassicae*. Sulphur, nitrogen, and potassium were varied in different experiments so as to produce: (a) plants showing deficiency symptoms, (b) plants growing normally, and (c) plants having pronounced vegetative vigor resulting from an extra supply of nitrogen or potassium. In comparison with the "complete" solution, the number of club-bearing susceptible plants was, in

general, increased slightly by an abundance of potassium, more by an abundance of nitrogen, and most by the absence of sulphur or nitrogen; it was decreased markedly by insufficient potassium. The number of resistant club-bearing plants was increased somewhat by high nitrogen, more by sulphur or nitrogen deficiency, and decreased by insufficient potassium. An abundance of potassium was not conclusive in its effect. On some otherwise healthy plants, a small gall, 1 or 2 mm. in diameter, appeared usually but not always at the base of a branch root. Fewer susceptible plants deficient in sulphur or nitrogen had galls. The number of resistant plants with galls was increased by the absence of sulphur or by high nitrogen and was decreased by high or low potassium. The behavior of the other nutrition series with respect to these overgrowths was not conclusive.

Control of Seed- and Soil-borne Diseases of the Potato. CHARLES S. REDDY AND G. N. DAVIS. Five potato varieties were used in an experiment to reduce virus diseases in potato seed lots to a minimum by repeated greenhouse indexing and increasing in isolated plots. These stocks were indexed each spring and grown on mineral soils in North Central Iowa. The percentage of virus-infected tubers decreased annually for 4 consecutive years (60 to 24 per cent), but increased the fifth to 45 per cent. The percentages of infected tubers for the 5-year period were 59.3, 31.5, 28.7, 24.0, and 44.7. No new seed stock was added during this period. However, 2 virus diseases, witches'-broom and calico mosaic, not present in the original stock, appeared the third year. It was concluded that, under Iowa conditions, the production of virus-free seed potatoes by greenhouse indexing is impractical. Efforts to find a better potato seed-piece treatment to control scab and *Rhizoctonia* have given data on yields when these diseases are largely controlled and on the fungicidal efficiency of different chemicals. It was found that the time requirement for treatment was influenced by surface tension of the liquid and that duration of treatment could be controlled by double dips, the second dip changing the nature of the fungicide. Data were obtained on the use of dust fungicides as potato-seed-stock treatments.

White Pine Selected in Blister-rust Areas. A. J. RIKER AND T. F. KOUBA. The invasion of unprotected Wisconsin areas by *Cronartium ribicola* has caused the death of most small white pine trees. Perhaps 1 out of 300 to 500 trees, however, survived several years in the midst of abundant natural inoculum and was apparently free from blister-rust infection. This circumstance suggested a search for young cone-bearing trees, without evidence of disease, in areas where rust had been severe for years. A number were found, and 163 such trees in four Wisconsin areas were selected during the falls of 1938 and 1939. These trees had survived natural inoculation for 15 to 20 years without any apparent cankers. Recent surveys showed approximately 10,000 to 60,000 feet of *Ribes* live stem per acre. It seems reasonable that some of these trees may be rust-resistant. Cones were collected in 1938 and 1939, and the seed from each tree was planted in separate rows in the nursery. Likewise, veneer grafts with scions from 40 parent trees were successfully made in the spring of 1939 and are now growing. When the seedling trees are old enough, it is planned to subject them and also grafts from the parents to artificial, as well as natural, inoculation.

Studies on Environmental Factors Affecting Infection and the Development of Bunt in Wheat. H. A. RODENHISER AND J. W. TAYLOR. Differences in percentages of bunt infection were obtained when wheat seedlings were grown in Hempsted silt loam from St. Paul, Minn., and in Mendon loam from Logan, Utah. Differences in effect were contingent, however, on incubation-period temperature, the effect of soil type being marked at 5° C. and not at 10° and 15°. Steam sterilization of Mendon loam effected uniform reduction in percentages of infection in Marquis wheat at each of these 3 incubation temperatures. Steam sterilization of Hempsted silt loam caused marked effect at 2 of the 3 incubation temperatures. At 5° it increased infection 63.7 per cent; at 10° there was no significant difference; and at 15° it decreased infection 58 per cent. Increases in infection were obtained with diseases in soil acidity from pH 4.8 to a point approaching neutrality. The most marked effect on infection was from pH 4.8 to 5.5. Increased bunt was obtained in Hope and Marquis with increased day length. Hope plants, exposed to light for 24, 10-11, and 8 hours daily, developed 64.1, 17.5, and 0.8 per cent of smut, respectively. The corresponding percentages for Marquis were 32.7, 17.8, and 1.9.

Properties and Purification of Alfalfa-mosaic Virus. A. FRANK ROSS AND W. M. STANLEY. Tobacco plants inoculated with alfalfa-mosaic virus show a rapid increase in the virus activity of their extracted juices until about the 10th day following inoculation. Subsequently, they show a sharp decline until the activity is less than one-tenth of the maximum reached. The virus is partially inactivated by freezing whole plants in a cold room held at -14° C. and to a lesser extent by rapid freezing with solid carbon dioxide. The extracted juice may be frozen by the latter method without markedly affecting virus

activity. The virus is inactivated less rapidly at 4° C. than at room temperature and apparently is unaffected by certain reducing systems. It is most stable between pH 6 and 7. When juice from diseased plants is centrifuged for 1½ hours at 4° C. in a field of approximately 60,000 times gravity, the supernatant liquid is essentially free of virus and most of the activity can be recovered by dissolving the pellets. Purified preparations containing protein, phosphorus, and carbohydrate have been obtained by means of differential centrifugation.

Physiological Specialization in Cercospora oryzae. T. C. RYKER. *Cercospora* leaf spot is the most serious disease of Blue Rose rice, the variety normally comprising about 75 per cent of the Louisiana acreage. Resistant varieties have appeared to be the most logical means of control. Several disease-free plants were collected in a heavily-diseased field of Blue Rose in 1936. From these a selection, 2854-3, was obtained that was resistant to *Cercospora* and at the same time indistinguishable from Blue Rose in type. However, in certain artificial inoculations made in 1939, this variety was completely susceptible, suggesting the possibility of more than one pathogenic race of the fungus. To test this, 4 varieties, Blue Rose, Fortuna, 2854-3, and Caloro were inoculated with 20 cultures of the fungus. At least three distinct groups were identified: group 1, to which Blue Rose was susceptible and the other 3 varieties resistant; group 2, to which 2854-3 was susceptible, Blue Rose moderately susceptible, and Caloro and Fortuna resistant; group 3, to which Blue Rose and Caloro were susceptible and 2854-3 and Fortuna resistant. Field observation suggests the occurrence of still additional forms. However, certain of the resistant varieties appear to be resistant to all strains of the fungus.

Seed Transmission of Tomato Mosaic. R. W. SAMSON. Commercial importance of seed transmission of tomato mosaic was suspected when 170 acres, contracted by one Indiana canning company for the production of tomato pulp and seed, was found to be 100 per cent infected with this disease. The acreage had been set with transplants grown from seed of mosaic-infected plants. The transplants were started in a greenhouse, transplanted to coldframes, and subsequently transplanted to the fields. Mosaic virus on seed saved from this acreage was demonstrated by rubbing water extracts of numerous samples onto leaves of Early Golden Cluster bean and Jimson weed. Brown, necrotic spots, identical with spots produced with diluted juice of tomato plants infected with mosaic virus, collected from plants in the canning acreage in question, developed on the rubbed leaves. No mosaic symptoms appeared on 10,000 seedlings grown to the 5th leaf stage from this seed. Seed transmission apparently did not occur. Several hundred acres, set with transplants from mosaic-infested seed from other sources and grown in greenhouses and coldframes, were found to have a high percentage of mosaic. Elimination of one or both of the usual transplanting operations appears to control the tomato mosaic commonly occurring in Indiana, as shown by the fact that several thousand acres of canning tomatoes, set with field-grown transplants or seeded directly, were found virtually mosaic-free, even though the seed came from mosaic-diseased plants.

Cultural Variation and Physiologic Specialization of Actinomyces scabies. LAWRENCE A. SCHAAAL.¹ Isolations of *Actinomyces scabies* were made from potato tubers from different geographic areas. Several cultural types often were obtained from individual pustules. Comparative studies of a large number of isolates grown on several nutrient media proved the existence of cultural races that differ in rate and type of growth, zonation, amount and color of pigmentation in the media, and in their tendency to sector. By inoculating differential varieties of potatoes many distinct parasitic races were recognized. *Actinomyces scabies* is unstable. Monosporous lines from certain cultures produced numerous sectors on potato-dextrose agar. Some of these variants continued to sector, yielding a variety of distinctly different cultural types. No line was completely stable, although a few lines produced only a single variant. It is possible that new parasitic races may arise from variants produced either in the soil or on the tubers. The existence of numerous parasitic races and the production of variants should be taken into consideration in breeding for resistance to scab.

Growth Stimulation of Diplodia zeae. G. SEMENIUK. On Czapek-Dox liquid medium, plain agar, and glucose agar, *Diplodia zeae* makes slow growth, while with additions of small amounts of water extracts of organic materials such as potato, carrot, cornmeal, oatmeal and pith of mature corn stalks, marked increases in growth rate follows. Stimulating properties were found in the aqueous solution of spore suspensions used for seeding purposes, which were obtained from cultures growing on whole-oat-extract agar. Such stimulating properties were not possessed by the liquid filtrates of

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31- or 36-day-old *D. zeae* cultures initially containing organic extracts or different nitrogen sources. Vitamin B₁ in amounts from 0.01 to 50 gamma per 25 cc. of liquid medium, had no effect, while white and yellow cornmeal were equally effective. The addition of these stimulating water extracts to the solid media increased pycnidial formation. Mycelial yields on Czapek-Dox medium with different inorganic nitrogen sources were greater in the presence of carrot decoction than in its absence. No marked effect was observed with bacto-peptone. The greatest stimulation occurred with thiourea, NaNO₂, and Ca(NO₃)₂. The NH₄ radical appears to be a readier source of nitrogen than the NO₃ radical.

Seedling Infection of Maize by Diplodia zeae in Steamed and Nonsteamed Soil. G. SEMENIUK. Equal amounts of *Diplodia zeae* inoculum were introduced into steamed and nonsteamed soil. The severity of corn seedling infection produced was from 10 to 50 per cent lower in the nonsteamed soil. Similar marked differences were obtained with field soil and compost, whereas only small differences existed between steamed and nonsteamed sand. Naturally infected seed was as severely diseased in nonsteamed as in steamed soil. In steamed soil containing inoculum, death of seedlings occurred at temperature intervals of 16-18, 20, 24-26, and 29-30° C. when the plants had attained a height of 2.0-6.0 cm. In nonsteamed soil containing inoculum, the seedlings approached the height of plants grown in soil without inoculum, even though the mesocotyl and roots were severely diseased. No marked temperature effects could be detected on seedling infection in nonsteamed soil. Maintaining seed in contact with inoculum in moist compost for 0, 2, 4, 6, and 10 days at 13.3° C. previous to transferring to a more favorable temperature for growth (20° C.) did not result in any greater infection in the nonsteamed soil. Delayed planting resulted in healthier seedlings. The antibiotic activity of other soil micro-organisms towards *D. zeae* may be the cause of the differences in the severity of infection observed.

The Prevalence and Destructiveness of Plant Diseases in Iowa from 1850 to 1937. D. R. SHEPHERD. In a study of records (dating back to 1858) of plant diseases in their relation to Iowa agriculture, 280, affecting 34 crops, were considered. Serious early losses from potato rot, thought to be late blight, were reported in 1858, 1865, 1866, 1869, 1876, 1885 and 1886. Fire blight of pear and apple became destructive in 1852 and did untold damage during the following 40 years. Consequently, much attention was focused on this and other orchard diseases. Early destructive outbreaks of rust of wheat (stem rust-leaf rust) were reported for 1859, 1876, 1878, 1880, 1890, 1893. Wheat scab caused widespread loss in 1865. Losses have resulted in the partial to complete elimination of some crops and a shift in acreage to those less vulnerable to attack. Stem rust, leaf rust, and scab appear to have been important contributing factors in the elimination of spring wheat as an Iowa crop; fire blight has been largely responsible for the almost complete elimination of pear culture; potato diseases in general have made potato culture in Iowa unprofitable; cabbage yellows ruined a thriving cabbage industry; and on Muscatine Island sand lands, watermelon wilt left only the remnant of a profitable watermelon industry in its wake.

Effect of Nitrogen Nutrition on Virus Multiplication in Tobacco. ERNEST L. SPENCER. The correlation previously reported between high-nitrogen nutrition and high virus concentration in Turkish tobacco plants (*Nicotiana tabacum*) has been found to be due to an increase in the rate of virus multiplication. Plants grown in nutrient sand cultures, supplied with a complete nutrient solution containing either 20 or 200 mg. of nitrogen per 100 cc., were inoculated with tobacco-mosaic virus when in the 3- or 4-leaf stage. At intervals, the juices of representative plants were assayed for virus concentration. On the 5th day after inoculation, little difference could be detected in the virus concentration of juices from the two sets of plants, but on successive days thereafter the virus concentration in juice from plants receiving 200 mg. of nitrogen became progressively higher than that in juice from plants receiving only 20 mg. of nitrogen. Since the two sets of plants grew at about the same rate, it is concluded that high-nitrogen nutrition increased the rate of multiplication of the virus.

Population Trends of Physiologic Races of Puccinia graminis tritici in the United States from 1930 to 1939. E. C. STAKMAN, R. C. CASSELL, AND W. Q. LOEGERING. There have been decided population shifts among physiologic races of *Puccinia graminis tritici* in the United States during the past 10 years, as determined by the percentage of uredial isolates of each race obtained from several hundred collections identified each year. Some races, such as race 38, have fluctuated greatly; others, such as race 34, increased gradually and then gradually declined (0.6 per cent in 1930; 22 per cent in 1934; and 0.6 per cent in 1939); some have declined to almost zero, notably race 36 (36 per cent in 1930 and 0.6 per cent in 1939); at least one, race 21, which constituted 7 per cent of

all isolates in 1934, was not found on wheat at all in 1939; still others, races 17 and 19, for example, have shown a general tendency to increase slowly but somewhat irregularly; and one, race 56, increased from 0.3 per cent in 1930 to 66 per cent in 1938 and 59 per cent in 1939. Indications are that temperature and other meteorologic factors may be important in these shifts. The implications of the facts in interpreting epidemics and in development and maintenance of resistant varieties are evident. (Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Potato Ring-rot Spread and Its Control by Disinfectants. G. H. STARR. Experiments conducted in 1939 at Laramie, Wyoming, showed corrosive sublimate to be the most effective, Mercurochrome relatively less effective, and acid-formaldehyde, Semesan Bel, Cinnex 20, formaldehyde, and lysol least effective as knife disinfectants for the control of spread of ring rot in potato tubers. After 2 days of healing-over, the percentage of ring rot, when compared with that in lots planted soon after cutting, was not reduced. In the treatment of potato tubers, both before and after cutting, Mercurochrome gave better control of ring rot than did Semesan Bel, Cinnex 20, or hot formaldehyde. There was 65 per cent of ring rot in seed treated before cutting, and but 40 per cent in that treated after cutting. The use of whole seed gave 23 per cent of ring rot, while slightly-infected seed gave 85 per cent. The cutting knife spread ring-rot infection to the 10th and last tuber of each lot. When the eyes of healthy tubers were smeared with ring-spot inoculum, infection occurred in 45 per cent of the plants. If inoculum was merely rubbed on the sound skin between the eyes, no infection resulted.

Treating Deciduous Trees for Chlorosis. G. H. STARR. Chlorosis of deciduous trees, cottonwoods in particular, has been especially severe during the past few years on the University of Wyoming campus and in many other parts of Wyoming. Injection of ferric phosphate in holes bored in the trunks of trees has given highly satisfactory results. Fifty-year-old trees, as well as younger ones, have responded to the treatment. What may be the correct amount to inject for successful results is still being tested. Five grams of ferric phosphate per inch-diameter of trees has given satisfactory results, but 5 times that dosage has been equally successful and perhaps its effect may last longer. Total leaf fall has resulted from summer treatments, but green leaves soon reappeared and obtained almost normal size before fall. Early-spring treatment of trees that appeared almost dead from severe chlorosis have been rejuvenated and restored to a normal green color. Several trees used as controls are now dead. The duration of treatment effect is not yet known, but previous results have shown that benefits may be expected for 4 or 5 years after treatment. Perhaps heavier dosages will increase this period.

Phloem Necrosis in the Ohio River Valley. ROGER U. SWINGLE. Phloem necrosis of elm continued in epidemic form during 1939. Although no extensive survey has been undertaken, the disease has been found in Ohio, Indiana, Illinois, Missouri, Tennessee, Kentucky, and West Virginia. To date it has been transmitted only by grafting. About 80 to 99 per cent of the patch grafts made union and about a year later 82 to 85 per cent of these showed the disease. In addition to the typically chronic cases of the disease reported previously, acute cases have been observed. In the former there is a gradual decline over a 12- to 18-month period before the tree dies; whereas, in the latter, apparently healthy and vigorous trees suddenly wilt and die within 3 or 4 weeks. During the past season 2 trees that expressed typical disease symptoms in previous years showed a marked degree of recovery. Seedling elms from one of these trees were extremely variable and, although none developed phloem necrosis, a few showed mosaic-like mottling of the foliage. Attempts to induce recovery of diseased trees by applications of the minor element boron and complete fertilizers have been unsuccessful.

Preemergence and Postemergence Factors that Influence the Infection of Barley by Covered Smut and Nigra Loose Smut. V. F. TAPKE. These two smuts, like the other seedling-infecting smuts of small grains, invade their host during seedling growth from the seed to the soil surface. Soil conditions during this preemergence period, especially moisture and temperature conditions, long have been considered the important factors affecting infection and the incidence of smut. In line with recent results with barley covered smut and oats loose smut, it was found that cold conditions, after the seedlings have emerged, also may result in marked reductions in the incidence of the nigra loose smut of barley. Temperate conditions for 10 days, 30 days, and continuously after seedling emergence resulted in progressively marked increases of smut. In another study, distinct increases in covered smut were obtained through impeding the subterranean growth of the seedlings by tamping or deepening the soil layer above the seed or by using a heavy soil. In another experiment, the incidence of covered smut was more than double when the early growth of fully-emerged seedlings was retarded through pruning the roots.

Control of Leaf Spot on Sour Cherries in West Virginia. CARLTON F. TAYLOR. Four applications of lime sulphur at 3 gal. of concentrate per 100 gal. of spray material is the accepted leaf-spot-control program on sour cherries in West Virginia. An experiment was conducted in 1939 to test the efficiency of this program. Each material under test was applied to 10 young (2-year) Montmorency trees arranged in single tree plots in randomized blocks. Sprays were applied on May 10 (petal-fall), May 18, June 12, and July 14-15 (post-harvest). The retained, uninjured leaves (on 1939 wood) on July 31 and September 15, respectively, were at the following percentage levels: 5 variations of lime sulphur averaged 88.7 and 5.0; phenothiazine, 1 lb. with bentonite and hydrated lime per 100 gal., 16.8 and 0.1; Bordeaux 3-2-100, 12.5 and 15.5; Bordeaux 3-5-100, 63.0 and 39.8; Copper Hydro, 3 lb. and 2 lb. of hydrated lime per 100 gal., 79.6 and 62.1; and nonsprayed checks (average of 5 plots) 0.3 and 0.0. Differences greater than 10.5 and 10.6 are probably significant at odds of 20:1. With the heavy infestation prevailing in this experiment, copper materials tested were much superior to lime sulphur in late-season retention of foliage.

Problems in the Determination of Physiologic Races of Ustilago avenae and U. levis. IAN W. TERVET. When susceptible varieties of oats were inoculated each year with different collections of oat smut, the percentage of smutted heads caused by some collections varied from year to year, while that caused by other collections remained constant for 6 years. One collection of *Ustilago levis* produced a relatively constant percentage of smutted heads on the susceptible variety Anthony for 6 years, but attacked Iogold lightly the first 2 years and very heavily the last 4. In 1937 a collection consisting mainly of *U. avenae*, with a slight admixture of *U. levis*, or a hybrid between these species, produced a moderate amount of smut on Anthony, Gopher, Iogold, and Black Mesdag. Spores of this collection produced on Black Mesdag were then used to inoculate these same 4 varieties in 1938 and 1939. Only Anthony and Black Mesdag became smutted, and the chlamydospores formed were characteristic of *U. levis*. Variation in these two cases can be ascribed to the selective effect of the variety from which the chlamydospores for inoculum were obtained. In the first case the host range of the collection was increased; in the second, the host range and the specific nature of the collection were changed.

Permeability Change as a Significant Factor in Parasitism. F. S. THATCHER. On susceptible wheat varieties *Puccinia graminis tritici* causes an increase in the permeability to water and to solutes of the host-cells of infected tissues. Resistance of Mindum wheat to race 36 is associated with an extreme localized decrease of host-cell permeability, which is considered to result in ultimate starvation of the pathogen. Increased susceptibility of Mindum wheat to race 36, as induced by narcotization, is related to increased permeability. Increased permeability precedes pectinase activity among soft rots, and is apparent in potato petiole tissue in a region beyond the zone occupied by mycelium of *Phytophthora infestans*. These facts explain certain characteristic symptoms of soft rots and late blight, and indicate an accessory rôle of permeability increase in the parasitism of the pathogens concerned. A decrease in the permeability of tissues of swede "root" near the margin of necrotic lesions caused by *Phoma lingam* was interpreted as being in accord with Brown's suggestion that a dry rot is determined by the ability of the host plant to restrict the amount of water reaching the parasite and, thus, arrest the progress of its enzyme activity at some intermediate stage. Wilt of excised tomato stems as induced by filtrates of cultures of *Fusarium lycopersici* was associated with increase in permeability of mesophyll cells to water. Wilting was not caused by death of xylem parenchyma cells associated with the conducting elements or by interference with the transpiration stream by pit closure. (McGill University. At present, Fellow Royal Society of Canada working in the Section of Plant Pathology, University Farm, St. Paul.)

Additional Facts Regarding Bacteriophage. ROY C. THOMAS. A nonspecific lysin, which is inactivated by heating at 56° C. for 30 minutes, has been found in many plants. When this lysin comes in contact with susceptible bacteria a change occurs resulting in the formation of a transmissible lytic principle, which is not inactivated at 60° C. and only partially at 65° C. This is believed to be the origin of the bacteriophage in plants and to function as a mechanism of resistance. These lytic principles vary with differences in cultures of bacteria used to produce them. In corn varieties susceptible to bacterial wilt, the lysin was lacking or very weak, whereas in resistant varieties it was strong. Several methods have been found effective in rendering cultures of *Aplanobacter stewartii* free of the lytic factor.

Inheritance of Resistance to Erysiphe graminis hordei in a Cross between Featherstone and Nepal Barley. J. S. TIDD. Greenhouse studies were made of the F₂ and F₃ of a cross between the barley varieties, Featherstone C.I. 1120 and Nepal C.I. 595. In addition

to being susceptible to race 6 of barley powdery mildew, Featherstone is awned, has a hulled caryopsis, and a short-haired rachilla. Nepal is resistant to race 6, is hooded, has a naked seed, and a long-haired rachilla. The F_2 plants were classified for these characters, and F_3 progeny tests also were made to check the F_2 -mildew classification. The data indicated that resistance was incompletely dominant, heterozygous F_2 plants being less resistant than the homozygous resistant plants. One main Mendelian factor is evidently responsible for the expression of resistance of Nepal to race 6. Mildew reaction also is inherited apparently independently of the other 3 character pairs studied in the cross, no linkage being found between any of the 4 factor pairs studied.

Verticillium Wilt of Chrysanthemums. PAUL E. TILFORD AND HARMON A. RUNNELS. Many varieties (423) of florists' chrysanthemums have been tested for resistance to *Verticillium* wilt. Those failing to develop symptoms and from which the fungus could not be isolated from the stems of plants grown in soil heavily infested with *Verticillium* were considered resistant. Roughly one-third (32.38 per cent) of the varieties proved resistant. Attempts to eliminate the fungus from cuttings by permitting them to take up fungicidal solutions have failed. Symptoms are most pronounced when the plants are in flower, and are least evident during periods of rapid vegetative growth. By roguing diseased plants when in flower, saving the first tip cuttings from the apparently healthy plants in the spring and growing the new plants in sterilized soil it has been possible to greatly reduce the amount of disease in a single season. Isolates of *Verticillium* from *Liatris*, soft maple, sugar maple, Norway maple, American elm, Japanese barberry and potato, although variable in culture, depending on cultural conditions, appear the same as isolates from chrysanthemum. Chrysanthemums, eggplants, and cinerarias were inoculated with most of these isolates. Some variability in virulence was observed but all were pathogenic.

Control of Leaf Blights of Fig. E. C. TIMS. Bordeaux mixture having proved ineffective in Louisiana as a control for thread blight of fig caused by *Corticium stevensii*, preliminary tests were made several years ago with some copper sulphate-lime arsenite eradicant sprays applied during the dormant season. These spray mixtures gave good results from the beginning, and in 1937 and 1938 gave almost complete control. Several trees sprayed with a copper sulphate-lime-zinc arsenite-monocalcium arsenite-fish oil mixture in 1938 remained free of thread blight during 1939, indicating that this spray mixture has a strong eradicant effect on the sclerotia of *C. stevensii*. The tests in 1939 were complicated by another leaf-blighting organism (*C. microsclerotia*), which caused very severe defoliation of many fig trees early in the season. While the above-mentioned spray mixture caused almost complete control of *C. microsclerotia* until the fig crop had been harvested in July, there was later some spread of the disease from adjoining non-sprayed trees to the sprayed area. This late infection probably was caused by sclerotia that developed in great numbers on the nonsprayed trees.

Observations on a Noteworthy Helminthosporium Disease of Corn. ARNOLD J. ULLSTRUP. The inbred line of corn Pr and two proprietary inbreds were observed to suffer from a severe attack by a species of *Helminthosporium* during 1938 and 1939. The symptoms on the leaves of Pr are characterized by numerous lesions ranging from 1 to 15 mm. in length. The lesions, generally oblong, because of partial delimitation by the veins, show faint zonation. Coalescence of lesions is common. The infected areas are at first a dead brown; later, with onset of fruiting of the fungus, becoming greenish gray. All aboveground parts are conspicuously attacked. Initial infection has been observed to take place in early summer. Under conditions of continued high humidity, fruition of the parasite is abundant and soon the entire plant is involved. Ears are attacked at any point and the mycelium may soon cover a large portion of the kernels. Morphologically, the fungus is distinct from *Helminthosporium turcicum* and *Cochliobolus heterostrophus*. The symptoms also differ from those produced by these species. *Helminthosporium zeicola* appears to be morphologically similar to the species in question. Symptoms indicated on the type specimen of *H. zeicola*, as well as reports on the pathogenicity of the latter do not suggest identical species. (Cooperative investigations of the Division of Cereal Crops and Diseases and the Purdue University Agricultural Experiment Station.)

Virus Distribution in Mosaic-susceptible and Mosaic-resistant Burley Tobacco. W. D. VALLEAU AND STEPHEN DIACHUN. Applying a white strain of the tobacco-mosaic virus to susceptible and resistant (Ambalema type) Burleys disclosed the fact that at the end of a month the distribution of virus within the plants was limited to chlorotic yellow patterns. Green areas, unless very close to yellow ones, were virus-free. The line between viruliferous and healthy tissue was more clearly marked in resistant plants than in susceptible, where invasion was more rapid. Mature, susceptible plants, inoculated at topping time

on the top and bottom leaves, respectively, developed mosaic in the suckers, and were extensively invaded in the top-inoculated leaves or were slightly invaded in the lower inoculated leaves. The remaining leaves, however, remained uninvaded for 30 days or more. After 30 days an occasional noninoculated leaf became slowly invaded along the base of the midrib, followed by a gradual spreading up and along the laterals. One striking difference between susceptible and resistant strains appears to be in the longer time required for the release of virus from infected areas of the resistant plants. There appears to be no barrier to rapid long-distance carriage of virus in highly resistant plants, once the virus is released from infected areas.

Classification and Nomenclature of Some Phytopathogenic Species of Bacillus. E. L. WALDEE, G. C. KENT AND I. E. MELHUS. Forty-six cultures of bacteria described as phytopathogenic species of *Bacillus* were studied according to the 1934 descriptive chart of the S. A. B. to determine their taxonomic status. From the preliminary data obtained, there emerge at least 3 well-defined groups of generic importance. Three species, formerly called *Bacillus amylovorus*, *B. tracheiphilus* and *Bacterium salicis*, constitute one group. The soft rot bacteria (24 isolates) constitute the second group. Two isolates designated as *B. lathyri* fall into a third group. Group I constitutes a generic group based on the type species, *Erwinia amylovora* (Burrill) Winslow *et al.*, 1917, and includes also *E. tracheiphila* (E. F. S.) Holland, 1920, and *E. salicis* (Day) Bergey *et al.*, 1939. Group II, comprising the soft-rot isolates, belongs to the coliform bacteria and should be classified with them in a new genus in the tribe Escherichiae (Bergey, 1939). Group III seems to contain 2 species of bacteria whose taxonomic position is still uncertain.

Histological Studies of Storage Scab Lesions on Mature Apple Fruits. E. A. WALKER. Two types of storage scab lesions were studied; i.e., continuation of growth around the margin of the prestorage scab lesion, and new scab lesions that developed while the fruit was in cold storage. Comparative studies were made with the storage and prestorage scab lesions on 4 varieties of apples. Storage scab lesions are somewhat sunken, due in part to the crushing and disintegration of the cortical cells under the mass of the scab organism. The fungus grows profusely through the cuticle tissue in a plate-like mass of pseudoparenchymous mycelium. The hyphae continue growth around the epidermal cells and may penetrate readily into the cortical tissue to a depth of 8 to 10 cells below the epidermis. The mycelium is found in the intercellular spaces, the middle lamellae, and is rarely intracellular. There is no cork or callus formation associated with the prestorage scab lesions; however, the cells immediately below the mass of scab mycelium are badly crushed and necrotic. The cuticle increased in thickness with increased length of storage; the scab fungus was seldom observed breaking through to produce conidia.

A Method of Reducing Clubroot Infection at Transplanting. J. C. WALKER, MARK A. STAHMANN AND DEAN E. PRYOR. The severest damage from clubroot (*Plasmodiophora brassicae*) on transplanted crucifers results from concentrated infection of the many rootlets that arise almost simultaneously from the lower stem and upper tap root after setting, especially since the application of water to facilitate recovery is also very favorable to infection. A weak solution of mercuric chloride used as the transplanting fluid greatly reduces infection in this zone, without injury to the host. In the search for a noncorrosive and more efficient fungicide several organic and inorganic compounds have been tested. Nothing superior to a solution of mercuric chloride in cost and efficiency has yet been secured. Use of the latter as the transplanting fluid in setting out cabbage on clubroot-infested soil gave commercially significant control on both mineral and muck soils in Wisconsin in 1938 and 1939. It is suggested that this method might well be tried in other regions. (University of Wisconsin and Division Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.)

Evidence of Passive Immunization of Plants from Curly Top. J. M. WALLACE. Following recovery from curly top, Turkish tobacco plants showed mild symptoms and were not visibly affected by reinoculation. Leaf-hopper transmission from recovered plants resulted in severe symptoms, indicating that the virus was unaltered in virulence. On the other hand, graft transmission resulted in mild symptoms. This indicated protective substances or properties in the recovered plants, transferable by grafting. Clons taken from leaf-hopper-inoculated plants at 5-day intervals following inoculation and grafted to healthy plants showed that a period of 20 days or longer was required for the inoculated plants to develop maximum effectiveness in affording protection. Further studies showed that plants of tomato varieties that seldom exhibit any ability to recover from curly top were provided with a partial protection when infected by grafting with recovered tobacco plants. Once established in the tomato plants, the protection was retained through several serial cuttings for more than a year and was transferable to

other healthy tomato and tobacco plants. This seems to be an example of a kind of passive immunization. This phenomenon has not been previously observed in plants.

New Facts Concerning the Plane Disease. J. M. WALTER AND P. V. MOOK. The plane disease has killed hundreds of planetrees in the Philadelphia and Baltimore areas. Recently, cases have been found in Newark, New Jersey, South Charleston, West Virginia, Magnolia, North Carolina, and Williamsburg, Virginia. Sufficient *Platanus acerifolia* trees of different sizes have been inoculated to demonstrate that the disease is caused by the *Ceratostomella* species that Jackson believed, from limited inoculation tests, to be the pathogen. Inoculation of 12 small seedlings of *Platanus occidentalis* resulted in development of typical symptoms. Trees affected by the disease in Vicksburg, Mississippi, South Charleston, West Virginia, and Williamsburg, Virginia, have been identified as American sycamore. The initial external symptom on recently exfoliated bark areas is a dark-brown or black discoloration, usually elongated in line with the grain of the underlying wood. On bark areas retaining older layers and scales, the first noticeable symptom is an elongate depression beneath which the inner bark is darkened. Infections occur on branches high in the crown, as well as in trunks. Branches flagging during August or September on trees whose trunks showed no lesions were found to have infections approximately 1 year old. Of 45 branch infections studied to date, 26 were associated with small pruning cuts. The fungus has been transmitted experimentally with pruning saws.

Pythium Injury of Oats. AARON WELCH. Oats, grown under Iowa conditions, are subject to serious root injury caused by *Pythium debaryanum*. It may cause a rotting of roots and of germinating seed. Under less favorable conditions for the pathogen, only local root lesions develop, which may cause stunting, yellowing, and, later, dying of the lower leaves of the host. The causal organism was easily isolated during the seedling stage. As the season advanced, however, isolation of *Pythium* became increasingly difficult. At maturity of the plant the pathogen was not isolated. It was found, however, that numerous secondary organisms rapidly followed *Pythium* infection. The secondary organisms were not isolated in the seedling stages, when *Pythium* was most prevalent. Under temperature-controlled greenhouse conditions 32 varieties of oats were tested in *Pythium*-infested soil. Germination was poor and root injury severe at 20° C. or below. Root development of the infected plants was suppressed, top growth reduced, and the development of secondary roots retarded. Infected plants yielded only half as much as the checks in terms of grain and total dry weight. Relatively few of the 32 varieties were resistant; most of them were susceptible.

Eradicant Sprays for the Control of Blossom Infection by Sclerotinia laxa. E. E. WILSON. Effect of arsenite sprays on development of sporodochia of *Sclerotinia laxa* in apricot and almond trees was again studied in 1938-39. Tests, conducted in 15 orchards in 8 counties, consisted of 120 plots containing 5 to 120 trees. The spray was applied in winter before sporodochia appeared on blighted hold-over twigs. Sodium and zinc arsenites were erratic or ineffective in preventing sporodochial development, but in its ability to kill sporodochia the former showed some promise when applied after these structures appeared. Calcium arsenite, on the other hand, frequently reduced development of sporodochia 95 to 99 per cent. In some of the small plots, despite efficient sporodochia suppression, blossom infection was abundant, possibly because spores drifted in from adjacent nonsprayed trees. In the larger plots, however, the amount of disease was reduced 65 to 95 per cent. Isolations indicated that calcium arsenite killed the fungus in a majority of the hold-over twigs. In others, though the fungus was not killed throughout, it failed to develop sporodochia. Almonds, in particular, have proved very sensitive to injury by arsenites. As a consequence, an important problem confronting the successful use of this type of control method is that of preventing serious host injury.

Comparisons of Phytomonas cerasi with Phytomonas syringae. E. E. WILSON. Although, in morphological and cultural tests, a number of workers proved *Phytomonas syringae* and *P. cerasi* to be closely related, the two species have not been widely accepted as identical. In the present studies they were indistinguishable in their utilization of sucrose, glucose, galactose, xylose, and the sodium salts of succinic, malic, citric, and lactic acids. Their nitrogen requirements were similar in that organic sources such as asparagin and peptone supported better growth than inorganic sources, such as ammonium nitrate and ammonium chloride. Other organic nitrogen compounds utilized to some extent were glycine, leucine, and tyrosine. When inoculated into dormant plum and cherry trees both organisms produced cankers similar in size and appearance. On the basis of this and other work it is proposed that *P. cerasi* and other species (*P. utiformica* and *P. prunicola*), earlier shown to be identical with it, be identified as *Phytomonas syringae* (Van Hall).

Comparative Values of the Fixed Coppers as Vegetable Sprays. J. D. WILSON AND H. C. YOUNG. From 8 to 14 of the so-called fixed or insoluble copper compounds now available were applied in the summer of 1939 to such crops as ginseng, tomatoes, celery, muskmelons, and beans. Notes were taken relative to the injury caused in certain instances, and comparative yields of the variously treated plots were obtained in most of the experiments. Rather definite indications of crop and disease specificity, with reference to injury and control factors, were observed for the different compounds compared. For instance, one of the materials, which gave good control of *Alternaria* blight of ginseng, ranked much lower with reference to the *Cercospora* leaf blights of carrot and celery, and another, which injured carrots severely, caused but little injury to tomatoes.

The Comparative Effect of Various Sulphur and Copper Sprays on Quality, Color, and Size of Sour Cherries. H. F. WINTER AND H. C. YOUNG. In this experiment lime sulphur, flotation sulphur, Bordeaux 1-2-100, 1½-3-100, 2-6-100, and 10 of the fixed coppers were compared. The fixed coppers were applied at the rate of 3 lb. to 100, based on 25 per cent metallic copper, with 3 lb. of hydrated lime. Cherries sprayed with the sulphur sprays were perceptibly lighter in color and contained less sugar and acids than those sprayed with copper. The sulphurs did not control leaf spot. Lime sulphur, 1½ gal. to 100, stunted the leaves. In general, the Bordeaux-sprayed cherries were smaller than those sprayed with fixed copper. Copper-sprayed cherries were darker and contained more sugar and acids than those not sprayed or sprayed with sulphur. Leaf spot was well controlled by most of the copper compounds.

The Diurnal Cycle of Taphrina deformans. C. E. YARWOOD. Uniformity of the asci and absence of expected intermediate stages of astus development in a given collection of curl-infected peach leaves suggested a diurnal cycle of *Taphrina deformans*. The cycle was followed by freehand and microtome sections of young material collected at different times of the day, by continuous collections of spores on slides exposed in infected trees, and by stimulating ascospore discharge from periodically collected leaves by means of the vapors from formalin-acetic acid-alcohol fixative. It was found that growth of the asci from the ascogenous cells began about 10 p.m. and reached full size about 3 a.m. By 6 a.m. there were usually 4 nuclei present in each ascus, and by noon the 8 ascospores were apparently mature. The maximum collection of naturally discharged ascospore groups was about 7 p.m., the minimum about 7 a.m. Exposure of infected leaves to the vapors of F.A.A. fixative induced ascospore discharge in greatest numbers in the afternoon and evening. The diurnal cycle was less marked, or was not apparent, on leaves on which asci had been maturing for several days.

Sporulation Injury Associated with Downy-mildew Infections. C. E. YARWOOD. Leaves infected with downy mildew of onions, downy mildew of spinach, and downy mildew of hops died sooner, or were more severely injured, if the fungus sporulated on the leaf surface than if such sporulation were prevented. The loss of green weight (as a quantitative measure of injury) due to a single night of sporulation was as much as 48 per cent of the original green weight for onions, 48 per cent for spinach, and 11 per cent for hops. The transpiration of leaves on which sporulation had just occurred was about 29 per cent less than that of nonsporulating infected leaves for onions and 28 per cent less for hops. The respiration of leaves on which sporulation had just occurred was not consistently different from that of nonsporulating infected leaves, but was about 47 per cent greater than healthy leaves on a green-weight basis, and amounted to about 0.23 mg. carbon dioxide per gram dry weight of leaves per hour in darkness at 19° C. Neither disturbed transpiration nor disturbed respiration is believed responsible for the sporulation injury observed, and the cause has not been determined.

Relative Susceptibility of Young Pine Trees in Artificial and Natural Stands to Invasion by Fungi and Bacteria. H. H. YORK. Investigations over a period of 10 years in forest plantings of white, red, and Scotch pines indicate that there is a very definite relation between the way in which trees are set in the ground and their susceptibility to infection in the root crown by fungi and bacteria. These studies, thus far, show that trees, established by natural seeding, are far less susceptible to invasion by these organisms.

Resistance of Tomato Varieties to Blossom-end Rot. P. A. YOUNG. With 4978 single-stem tomato plants per acre, serious commercial loss resulted from an average of only one rotted fruit per plant. Extensive field tests at Jacksonville, Texas, from 1937 to 1939 showed large variation in resistance to blossom-end rot among different tomato varieties and their selections. Most selections of Marglobe, Break O'Day, and Pritchard were resistant. Varieties tested were classified in 3 groups based on the range in average numbers of fruits with blossom-end rot per plant. The resistant group with 0 to 0.8

fruits with blossom-end rot per plant included Blair Forcing, Break O'Day, Globe, Long Calyx Forcing, Marglobe, Marhio, Michigan State, Norton, Pritchard, Sureset Forcing, and Tennessee Red. Moderately susceptible group with 0.9 to 2.0 fruits with blossom-end rot per plant included Baltimore, Century, Early Baltimore, Glovel, Grothens Red Globe, Gulf State Market, Illinois Baltimore, Kanora, Louisiana Pink, Marvana, Marvel, Newport, and Sweetmeat. Very susceptible group with 2.1 to 6.5 fruits with blossom-end rot per plant included Browns Special, Buckeye State, Globelle, Illinois Pride, Louisiana Dixie, Louisiana Red, Prairiana, Riverside, and Rutgers. Commercial strains resistant to blossom-end rot were selected from Rutgers and other valuable varieties.

RELATION OF WOUNDS TO INFECTION OF AMERICAN ELM BY CERATOSTOMELLA ULMI, AND THE OCCURRENCE OF SPORES IN RAINWATER

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INTRODUCTION

Reports indicate that *Ceratostomella ulmi* (Schwarz) Buisman, causal fungus of the Dutch elm disease, may fruit in various places on field and planted elm under natural field conditions. In Europe, Fransen (5), Roepeke (12), Wollenweber (20), and others observed fruiting structures of this fungus in maternal galleries and pupal cells made in diseased elm by certain scolytid beetles. Buisman (3) saw perithecia of *C. ulmi* that had developed "in the bark" of a standing diseased elm. In England, Peace (10) reported *C. ulmi* fruiting on dead wood in "sheltered places such as cracks between bark and wood," but he added that such fruiting was not commonly seen. May and Gravatt (7) stated that, under very favorable conditions, spores were formed on the cut surfaces of diseased trees and stumps and in insect tunnels in the wood. Later, May (8) observed coremia on the ends of logs cut from diseased trees and under the loose bark of old logs lying on the ground.

In field observations in and near New York City during the past 4 years the writers found that coremia of *Ceratostomella ulmi* are often produced in more or less exposed places on elms infected by the Dutch elm-disease pathogen and on decadent trees, apparently not infected by this fungus. Coremia were seen on the outer surface of the inner bark and they protruded into the space created by the loosened and partly raised outer rough bark. Sometimes this outer bark was sloughed off so that the coremia were fully exposed. Coremia were observed in the openings into leopard-moth galleries, and in scolytid entrance holes on diseased trees. They developed in scolytid feeding wounds on twigs taken from healthy trees in the field and held in glass moist chambers in the laboratory. Also, they developed frequently in artificial wounds and in scolytid feeding wounds made on experimental trees held under high moisture conditions in the greenhouse.

¹ The writers are indebted to Dr. D. S. Welch, for suggestions concerning experimental procedure, and for critically reading the manuscript. Thanks are due Dr. W. H. Rankin, New York State Department of Agriculture and Markets, for helpful cooperation in some of the field work; and the Boyce Thompson Institute for Plant Research for making available laboratory space and equipment.

Several workers have suggested the possibility of wind dissemination of the spores of *Ceratostomella ulmi* in the field, but all were doubtful of its importance (2, 5, 7, 8, 10, 12, 20). Smucker (14), in the United States, reported that spores could be dislodged from cultures and carried for some distance by air currents in the laboratory, but attempts by Fransen (6), in the Netherlands, to dislodge spores by means of air currents were unsuccessful.

The writers' experiments showed that spores were sometimes dislodged and carried by air currents. This seemed dependent on the age and moisture content of the cultures used, and on the velocity of the air currents (the velocities were measured with an anemometer). The so-called "Cephalosporium" stage of *Ceratostomella ulmi* was present on all cultures used in this work, and it was noted that bits of mycelium were almost or quite as easily torn away from such cultures as were the spores themselves. The results show further that, always, only a few spores are dislodged regardless of the conditions prevailing in the different experiments. Available data are insufficient to determine the optimum conditions for dislodgment of spores by means of dry air currents. Further work is needed to help clarify this question.

Wollenweber (20) suggested that the Dutch elm-disease fungus might be disseminated by water. The writers found that large numbers of *Ceratostomella ulmi* spores could be dislodged from cultures by means of atomized water. The cultures from which spores were dislodged contained both coremia and conidiophores growing on sterilized elm twigs. Dislodged spores were carried in small droplets of water and many were caught on nutrient agar in dishes distributed at distances of immediately below to 2 to 3 feet away from the source of inoculum. Comparison of the results obtained from attempts to dislodge spores by means of dry air currents and by means of water showed that, in the laboratory experiments, water was decidedly more effective as a disseminating agent of this fungus. Because of the ease with which spores were dislodged by water it appeared that rainwater might wash them from the fruiting structures of *C. ulmi*, formed in more exposed places on elm in the field. Therefore, rainwater was collected from diseased and apparently healthy field elms and tested for presence of this organism.

ISOLATION OF CERATOSTOMELLA ULMI FROM RAINWATER

During the summer months of 1937 and 1938, rainwater was collected from a total of 32 diseased and 8 healthy elms located in Westchester County, New York.² The water was caught by means of special apparatus attached to the tree trunks near their bases. One to 11 liters of water were caught and collected from each tree. This water was brought to the laboratory and centrifuged. Small samples of the material concentrated on the bottom of the centrifuge bottles were withdrawn with a sterilized pipette and mixed with nutrient agar in Petri dishes. After incubation at room temperature

² George E. Thompson, now of the Department of Plant Pathology and Plant Breeding, University of Georgia, did the necessary field work during the summer of 1937.

for several days the dish cultures showed that *Ceratostomella ulmi* was present in water collected from 9 of 32 diseased trees, but was not present in the water collected from the 8 apparently healthy trees. The number of *C. ulmi* colonies obtained per 2 cc. sample of centrifuge concentrate ranged from 1 to 39. Since this sample represented only a small part of the total volume of concentrate per liter of water, it is obvious that considerable numbers of spores were present in water from some of the trees.

Possible sources of *Ceratostomella ulmi* spores found in rainwater, which was collected from diseased trees are as follows: (a) fruiting structures formed in more or less exposed places; (b) frass pushed out by insects during construction of their egg galleries; (c) spores deposited by *C. ulmi*-infested insects, such as *Scolytus multistriatus* Marsh., *Hylurgopinus rufipes* Eich., *Saperda tridentata* Oliv., *Magdalis barbata* Say, and *M. armicollis* Say, during their visitations to and activities in diseased trees; and (d) in undetected perithecia, although as far as known such fruiting structures have never been seen on diseased trees in the field in the United States.

INTRODUCTION OF CERATOSTOMELLA ULMI SPORES INTO WOUNDS ON AMERICAN ELM

During the past 4 years many kinds of wounds were tested as infection courts, on different parts of 3- to 4-year-old budded elms (*Ulmus americana* L.) potted in pails. Based on parts of trees injured and on the extent of the injuries, 4 classes of wounds were tested.

Class 1.—Wounds which penetrated but did not extend through the cortical layers. Such wounds were made in bark of (a) current-year shoots, (b) 1-year-old branches, and (c) 2-year-old bark of trunks. The wounds were made by cutting transversely into the bark with a knife, then stripping the outer bark downward from the cut so that the inner cortical layers were exposed. When such injuries were made the wood underneath was never injured or exposed.

Class 2.—Wounds that exposed the outermost surface of the wood in roots. Longitudinal incisions were first made with a knife in the bark of branch-roots and of main-roots, care being taken that such incisions did not reach the wood. The bark on one side of the incision was pryed loose exposing the uninjured surface of the wood and the spore suspension was then introduced under the bark with an atomizer. Inoculum was thus applied directly to the uninjured surface of the wood.

Class 3.—Wounds that exposed and injured the wood of roots, stems, and branches. Such wounds consisted of (a) slanting cuts made with a knife on trunks and branches; (b) *Scolytus multistriatus* feeding wounds; (c) fresh pruning wounds; (d) old pruning wounds; (e) small branches twisted at their bases so that the bark was split and the wood injured and exposed; (f) broken shoots and branches; and (g) cuts made with a one-half-inch wood chisel on the roots.

Class 4.—Wounds that exposed and injured the xylem elements of newly formed or forming parts of green and succulent shoots and leaves.

Such wounds were made by (a) vigorously whipping together the tree-tops so that leaves and green, succulent shoots were abraded and broken; (b) detaching leaves so that leaf traces were exposed; and by (c) puncturing leaf midribs with a needle.

With the exception of root wounded trees included in class 3, 4 trees were used to test each kind of wound on a given date. At least 1 test was made of each kind of wound during the active growth period³ of the trees, and repeated once or twice on different groups of trees after terminal growth had ceased. This was done to study the effect of introducing *Ceratostomella ulmi* into different kinds of wounds during different stages of tree growth. Each kind of wound on aboveground parts was tested on 11 to 28 trees in experiments done during the 4-year period. The numbers of wounds per tree ranged from 5 to 15, depending on the kind and means by which they were made.

The relation of humidity to infection of elms by *Ceratostomella ulmi*, through wounds on aboveground parts, also was studied.

Root wound tests were started early in April, when the buds had begun to swell, and repeated on different groups of trees (3 to 12 trees per group) at intervals of 1 week to 1 month until early November. Wounds were made on the roots usually by piercing the soil around each tree several times with a one-half-inch wood chisel. The number of such wounds per tree was not determined.

Three methods of applying the inoculum were used: 1. *Ceratostomella ulmi* spores were suspended in water and atomized directly on wounds. 2. Spores were dislodged from cultures by means of water spray. In this method the spray from an atomizer containing water was forced past coremia and conidiophores that developed on autoclaved elm twigs previously planted with the fungus. Twig cultures were held above the trees and 12 inches outside the crown periphery. As the water spray came in contact with the fruiting structures, spores were dislodged and carried in droplets to wounds on the trees. 3. Root wounds, included in class 3, were exposed to inoculum by infiltrating 500 cc. of a water suspension of spores into the soil around each potted tree. Care was taken, when any one of the methods was used, to distribute approximately equal amounts of inoculum to each tree. Except for old pruning wounds, the inoculum was always introduced into the wounds as soon as they were made.

Percentages of wilt and dieback were recorded at short intervals for each of the trees until they were cut, which was usually in the fall of the year they were inoculated. Some were held for observation in the following year after which they were cut. When cut the trees were carefully examined for

³ The term "active growth period," as used in this paper, denotes that period beginning (in potted trees used in these experiments) when the new shoots reach 2 to 4 inches in length and continuing until extension in shoot length ceases. This period begins 4 to 5 weeks after the leaf buds begin to open and ends about 2 months later. It is during this period that American elms appear to be most susceptible to infection and invasion by *Ceratostomella ulmi*. However, in trees becoming infected during the latter part of this period there is a definite tendency toward less extensive invasion by the fungus.

the brown discoloration commonly caused by *Ceratostomella ulmi*. The recovery of this fungus from discolored wood was used as the criterion of infection.

The results obtained from introducing *Ceratostomella ulmi* spores into wounds made on small elms are given in table 1. In general, these results agree with those reported by other workers (1, 4, 9, 13, 15, 17, 18, 19). The data for each wound class are discussed separately.

Class 1.—Infection was not obtained in trees by introducing spores into wounds made in cortical tissues that did not reach the wood. Radulescu (11) concluded, with some reservation, that infection could result from introducing the Dutch elm-disease fungus into bark wounds that did not injure the wood. The writers observed that injuries to the bark sometimes resulted in splitting that produced fissures in the inner bark tissue beneath the wounds soon after they were made. This splitting seemed partly attributable to pressure exerted on the injured bark due to normal radial growth of the injured plant part. Data in table 1 show that infection occurred in 5 trees; but in each of these cases it was observed that the inner-bark tissue covering the infected wood had been split. Apparently the fungus had, in these cases, reached the wood through fissures produced by splitting. In the remaining trees the inner bark underneath wounds did not split, and the fungus did not penetrate the intact tissue, at least not sufficiently to reach and establish itself in the wood.

The wounded bark tissue in each wound was excised and planted on nutrient agar in Petri dishes when the trees were cut. *Ceratostomella ulmi* was recovered from very few of these tissue plantings. Recovery of the fungus showed that it lived at least 3 months in the bark and during this time it apparently was unable to penetrate to the wood.

Class 2.—The number of trees that became infected through direct application of spores to the uninjured surface of the wood in roots was high, although the infected trees were not always invaded to the same extent. All trees inoculated on April 25, before the active growth period began, were infected, but only 1 was extensively invaded, the others showing mere traces of discoloration in the wood at the inoculation points. All trees inoculated during their period of active growth (June 2 and July 13) were infected and three-fourths were extensively invaded. Of those trees inoculated on August 22, which was about 5 weeks after terminal growth had ceased, 1 became infected and developed only a trace of discoloration.

Class 3.—With the exception of old pruning wounds, other wounds in this class readily admitted *Ceratostomella ulmi*. Old pruning wounds, having aged 3 months or more when the fungus was introduced into them, apparently had healed sufficiently during this time to entirely prevent entrance of the fungus. The incidence of infection through favorable wounds appeared to depend somewhat on the inoculation method (Table 1). The growth stage of trees at time of inoculation exerted a marked effect on the extent of subsequent invasion. The effect of the growth factor is discussed elsewhere in this paper.

TABLE 1.—Results of introducing *Ceratostomella ulmi* into wounds made on American elm

Wounds ^a		Date; numbers of trees inoculated and infected ^b						
Class	Kind	1935	1936	1937		1938		
1	Green bark			<i>June 14</i> 4/0	<i>Aug. 5</i> 4/2c	<i>May 27</i> 4/0	<i>Aug. 23</i> 4/0	
	One-year-old bark			4/0	4/1c	4/0	4/0	
	Two-year-old bark			4/2c	4/0	4/0	4/0	
2	Wood of roots exposed but not injured			<i>July 13</i> 4/4		<i>Apr. 25</i> 4/4	<i>June 2</i> 4/4	
							<i>Aug. 22</i> 4/1	
3	Slanting cuts		<i>June 24</i> 4/3	<i>Aug. 12</i> 4/3	<i>June 12-14</i> 4a/4	<i>July 26</i> 4a/2		
	Scolytid feeding				4a/3	3a/2	<i>May 13</i> 4/4	
	Fresh pruning		4/4	4/2	4a/3	4a/4		
	Old pruning		4/0	4/0	4/0	4/0		
	Basal, twisted branches	<i>July 16</i> 4/3	<i>July 30</i> 4/3	<i>Sept. 9</i> 4/4				
	Broken branches	4/3	4/4	4/4	4a/1	4a/1	4a/2	
Wood of roots exposed and injured ^c		1937						
		<i>Apr. 6</i> 4/2	<i>Apr. 17</i> 4/1	<i>May 4</i> 4/3	<i>May 25</i> 4/4	<i>June 18</i> 3/3	<i>July 13</i> 8/8	<i>Sept. 1</i> 4/4

TABLE 1.—(Continued)

Class	Wounds ^a		Date; numbers of trees inoculated and infected ^b			
	Kind		1935	1936	1937	1938
4	Whipped foliage		July 16 4/0	Sept. 9 4/0		
	Leaf scars and traces			June 24 4/3	Aug. 12 4/2	June 14 4/1
	Punctured leaf mid- ribs		4/0	4/1	4/0	4/1

^a See text for description.^b Inoculation was by method no. 1 (see text) except as otherwise noted below by footnotes ^d and ^e. Inoculated and infected trees expressed as a fraction: numerator = trees wounded and inoculated; denominator = trees infected. The recovery of *C. ulmi* from discolored wood was used as the criterion of infection.^c Injured bark had split due to radial growth; see text for explanation.^d Inoculated by means of method no. 2; see text for description.^e Inoculated by means of method no. 3; see text for description.

Class 4.—Data given in table 1 show that leaf traces were favorable for the entrance of the fungus. Infection very infrequently resulted from spores introduced into punctures made in leaf midribs, and into abrasions made on succulent parts of growing shoots. Trees infected from spores introduced into wounds of this class never were extensively invaded; the fungus always remained localized and none of the trees wilted.

Non-wounded trees were inoculated and included in each experiment as checks. The 3 methods of inoculation were tested on such trees, but none of these check trees became infected.

Briefly summarized, the data show that infection never resulted in trees through non-wounded surfaces. Infection was not obtained through injured cortical tissues that did not reach the wood, although *Ceratostomella ulmi* was reisolated from injured bark tissue several months subsequent to inoculation. Infection was obtained through the intact surface of freshly exposed wood, but invasion was more extensive in trees inoculated during their active growth period than in those inoculated before or after this period. Infection was easily obtained by introducing spores into wounds that exposed and injured the xylem elements of roots, stems, branches, and mature parts of new shoots. Infection sometimes resulted from introducing spores through injuries on leaf midribs, leaf traces, and through injuries on succulent parts of young shoots, but the fungus remained localized and never caused wilt. Wounds particularly favorable for the entrance of *C. ulmi* were: root wounds that extended into the wood; slanting cuts on trunks and branches; fresh pruning wounds; broken, and twisted branches; and *Scolytus multistriatus* feeding wounds.

The 3 methods of inoculation used were generally successful, although the direct method of introducing spores into wounds on aboveground parts by atomizing them with a water suspension of spores was somewhat more sure than the method whereby spores were dislodged from cultures with and carried to wounds by a water spray. Data in table 1 show that the incidence of infection in trees inoculated by the second method sometimes was not so great as in those inoculated by the first method. This difference was more obvious when placed on the basis of total wounds involved. Undoubtedly this was due to the accumulation of a greater number of spores per wound by the first method, resulting in a more pronounced effect from the mass attack. Inoculation accomplished by the third method of placing spores in the soil around wounded roots (Class 3) was particularly successful. This method resulted in a high incidence of infection and usually in extensive invasion, although the extent of the latter depended largely on the growth stage of trees at the time of inoculation.

It has been demonstrated that spores of *Ceratostomella ulmi* may be introduced into American elm, through wounds that expose xylem elements and thereby cause infection. Also it has been shown that spores of this pathogen are sometimes present in the "run-off" water from diseased elms. Since wounds are commonly present on various parts of field and planted elms, it

seems obvious that spores may sometimes be introduced into them by means of water. *C. ulmi* spores suspended in water and infiltrated into soil around the roots of potted elms caused infection through wounds made on the roots as long as 2 weeks after the spores were originally placed in the soil. Furthermore, Verrall (16) reported that *C. ulmi* survived sparingly in non-sterilized forest humus *in vitro* as long as 3 months.

FACTORS INFLUENCING THE OUTCOME OF INOCULATION

Stage of Growth

Infection was obtained by introducing spores of *Ceratostomella ulmi* into the soil around wounded roots at any time from April 2 to November 3, inclusive (Table 1, Class 3). The percentage of trees becoming infected was greatest in those inoculated during the period between May 25 to September 1, inclusive. Further, there was great variability in the extent of invasion resulting from inoculation and infection at different times between April 2 and November 1. Trees inoculated before buds began to swell and up to the time new shoots had reached 1 to 3 inches in length (the latter figures represent the maximum length of new shoots on trees inoculated on May 4) were mostly infected but seldom if ever severely diseased. Discoloration in the wood of such trees caused by *C. ulmi* was always scanty and usually in the annual ring formed in the preceding year. The invasion, which followed inoculation and infection on and after May 25 until terminal growth of shoots had ceased (terminal growth was completed by July 1 to 15), was extensive and most of the trees were severely diseased; this is based upon leaf wilt, dieback, and fungous discoloration. Trees inoculated within the period between cessation of terminal growth and early September often became extensively invaded, as shown by fungous discoloration, but they died back very little and most of them grew well during the following year. Most of the trees inoculated in October and early November were infected but the invasion resulting was incipient.

Data given in table 1, classes 3 and 4, show that inoculation of trees on different dates did not significantly affect the incidence of infection through different kinds of wounds, except for root wounds. For example, spores introduced into fresh slanting cuts on different trees, on June 24 and August 12, by atomizing them with spores, resulted in equal numbers of infected trees on the 2 dates. Infection results obtained from introducing spores into other kinds of wounds in these classes, by the same method, are similar and they substantiate the statement that the date of inoculation, or rather the growth stages (within the date limits of these inoculations) did not greatly influence the incidence of infection. However, there was great variability in the extent of invasion following inoculation and infection of trees during the different months. In general, extreme invasion, followed by severe dieback, resulted from introducing *Ceratostomella ulmi* into any kind of wound on aboveground parts (except bark wounds that did not reach the

wood, wounds on leaves, those on succulent parts of green shoots, and exposed leaf traces) during the active growth period of the trees. Subsequent inoculations done until the end of August generally resulted in less extensive invasion and the trees seldom died back. Invasion of such trees also was variable, as shown by discoloration, and depended to some extent upon the kind of wound used as an infection court. Invasion was more extensive when the inoculum was introduced through slanting cuts in trunk and branches, broken branches, fresh pruning wounds, and *Scolytus multi-striatus* feeding wounds than it was when the inoculum was introduced into wounds made by twisting branches, into leaf traces, wounds on leaves, and into other wounds which exposed but did not injure the xylem elements.

Humidity

In all wound experiments, except those involving root wounds, some trees from each wound-group were placed on greenhouse benches immediately following inoculation, and their foliage and branches were always kept dry. Other trees from the same groups were held in a greenhouse moist chamber for 10 days immediately following inoculation and then removed and placed on greenhouse benches; thereafter, their foliage and branches also were kept dry. The relative humidity maintained in the moist chamber ranged predominantly from 85 to 98 per cent, while that of the greenhouse, with ventilators constantly open, ranged predominantly from 40 to 80 per cent. Wounded tissue on trees held in the moist chamber remained moist throughout the 10-day treatment, while that on trees in the open greenhouse rapidly became dry. Infection was obtained more frequently in trees placed in the moist chamber than in those exposed constantly in the open greenhouse. This difference was evident in all experiments involving the introduction of *Ceratostomella ulmi* spores into different kinds of wounds, on aboveground parts, which were most suitable for entrance of the fungus. These inoculations were made from May through August. The moist-chamber treatment did not appear to influence the extent of invasion, since trees becoming infected under this condition were invaded to about the same extent as those held under drier conditions.

The moist-chamber treatment very markedly affected the ability of *Ceratostomella ulmi* to fruit in the wounds. On trees exposed in the moist chamber, coremia were present in all types of wounds made on aboveground parts, except those made on leaves and on succulent parts of green shoots, but there were none in those wounds on trees held constantly in the open greenhouse.

RELATION OF WOUND HEALING TO INFECTION

It has been shown that infection may result from introducing *Ceratostomella ulmi* spores into many kinds of wounds freshly made on small elms. To study the relation of wound healing to infection, spores were introduced into wounds after varied periods of aging and healing. Three kinds of wounds were used: (a) slanting cuts made with a knife on stems and on

branches; (b) feeding wounds made by surface-disinfected *Scolytus multi-striatus* beetles; and (c) cuts made on roots by piercing the surrounding soil with a $\frac{1}{2}$ -in. wood chisel. After the trees were wounded they were held outdoors to allow their wounds to heal under more nearly natural conditions. Trees having wounds on aboveground parts were moved into a cheesecloth-screened greenhouse just before inoculation, where they remained until cut and examined. Each tree was carefully examined before being inoculated to make sure that wounds other than those made for the test were not present.

Some trees with beetle wounds and some with knife wounds were brought to the greenhouse and inoculated on the day wounds were made. Since beetles fed 3 days before being removed from the trees, some feeding wounds were as much as 3 days old when the first trees were inoculated. Thereafter, different trees with wounds were brought to the greenhouse and inoculated at intervals of 3, 6, 13, and 27 days subsequent to the day they were wounded. Trees having wounds on aboveground parts were inoculated by atomizing a water suspension of spores directly upon the wounds. Following inoculation, each group of trees was divided; some of the trees were placed on greenhouse benches where their branches and foliage were always kept dry; the others were held on benches in the same greenhouse and their branches and foliage sprinkled lightly with water twice daily for 7 days, after which they were treated like those always kept dry.

By means of the soil-infestation method, some of the root-wounded trees were inoculated on the day wounds were made; others at intervals of 1, 3, 7, 14, and 30 days subsequent to being wounded. After inoculation these trees were held in an outdoor cage made of wire screen.

All trees were wounded and inoculations completed within the period of their active growth. Notes on wilt and dieback in each tree were taken at short intervals. The trees were cut in the autumn of the year they were inoculated, and each tree was examined in detail to ascertain the presence or absence of fungous discoloration. The isolation of *Ceratostomella ulmi* from discolored tissue was used as the criterion of infection.

The results definitely show that, for knife wounds and for beetle-feeding wounds, the chances for infection became progressively less as the interval allowed for healing of wounds was increased. All trees inoculated during the first 6 days were infected and most of them were extensively invaded, while others inoculated at the end of the 13-day interval were likewise all infected but only 1 was extensively invaded. Infection was slight following the introduction of spores into wounds after 27 days. The fungus gained entrance through only 20 per cent of the wounds into which spores were introduced at this time; the resulting discoloration from which *Ceratostomella ulmi* was isolated showed that the fungus had progressed not more than 1 to 2 cm. in the tissue around these wounds. Briefly, it appears that beetle and knife wounds made on small trees require somewhat more than 27 days for healing to entirely prevent infection. Infection followed by extensive invasion may be expected occasionally from the introduction of spores into 2-week-old wounds and frequently when the wounds are less than 2 weeks old.

The effect of sprinkling was noticeable only in trees inoculated at the end of the 27-day interval. Knife-wounded trees that were not sprinkled were not infected, while some of the sprinkled ones were.

The results obtained from introducing *Ceratostomella ulmi* spores into root wounds of different ages were somewhat similar to those for wounds of different ages on aboveground parts. Root wounds up to 1 week old markedly favored fungal entry; all trees were infected and most of them were extensively invaded. Trees with 2-week-old wounds were mostly infected but none were extensively invaded, while trees with 1-month-old wounds were not infected.

SUMMARY

Field observations show that coremia of *Ceratostomella ulmi* are often produced in various places on field and planted elms infected by this organism, and on decadent elms apparently not infected by this fungus. Coremia in some of these places are sometimes fully exposed to the outside.

In laboratory experiments, *Ceratostomella ulmi* spores were easily dislodged from fruiting structures by means of a water spray, but spores were not easily dislodged by means of dry air currents.

During the summer months of 1937 and 1938 rainwater was collected from a total of 32 diseased field elms; water from 9 of these yielded *Ceratostomella ulmi*.

Infection was easily obtained in potted American elms as a result of introducing *Ceratostomella ulmi* spores into fresh wounds that extended into the wood of roots, trunks, and branches. Infection also was obtained when inoculum was applied to the surface of wood freshly exposed but uninjured. Infection sometimes was obtained when spores were introduced into leaf traces, and into injuries on leaf midribs and succulent parts of newly formed or forming shoots; but the resulting invasion was always slight, and the trees did not wilt. Infection did not result from applying spores to non-wounded surfaces; neither did infection result from introducing spores into cortical wounds that did not reach the wood.

Data are discussed showing that the aging and healing of wounds made on potted elms had a marked effect upon the entrance of *Ceratostomella ulmi*.

Potted trees, inoculated at any time within their active growth period, were extensively invaded; many of them died back considerably. Trees inoculated before this period frequently became infected, but invasion by the fungus usually was slight. Trees inoculated after this period usually became infected, and some were extensively invaded; these died back very little or not at all.

Infection occurred more frequently in trees, inoculated through wounds on aboveground parts, which were held under high moisture conditions, than in other trees similarly inoculated but held under drier conditions. Constant high moisture conditions favored the development of coremia in wounds.

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ECOLOGICAL SPECIALIZATION IN THE STEM- AND BULB-
INFESTING NEMATODE, DITYLENCHUS DIPSACI
VAR. AMSINCKIAE¹

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INTRODUCTION

Steiner and Scott (10) have reported for various points in California the occurrence of a nematosis of *Amsinckia intermedia* Fish. and Mey., a very

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common annual herbaceous wild flower of central California. The mature-plant symptoms are described as "greatly enlarged fruits, transformed by the parasite into obvious galls." It is stated that "All portions of the plant above ground may harbor the nema." Observations made by the writer in the spring of 1933 and subsequently have disclosed that up to the end of the growing season there is no tissue infestation, except in the fruits. Leaves, bracts of various kinds, and stems are entirely free, except for occasional migratory larvae found only on the surface. This evidence of marked tissue specialization, unusual for *Ditylenchus dipsaci*, stimulated research into the nature of the relations of the nematode to the host plant. Consequently, in the spring of 1936, infested plants were studied in every stage of development from emergence to complete maturity. The results of these studies are here reported. Figure 1 illustrates the typical symptoms of the disease in the mature plant.

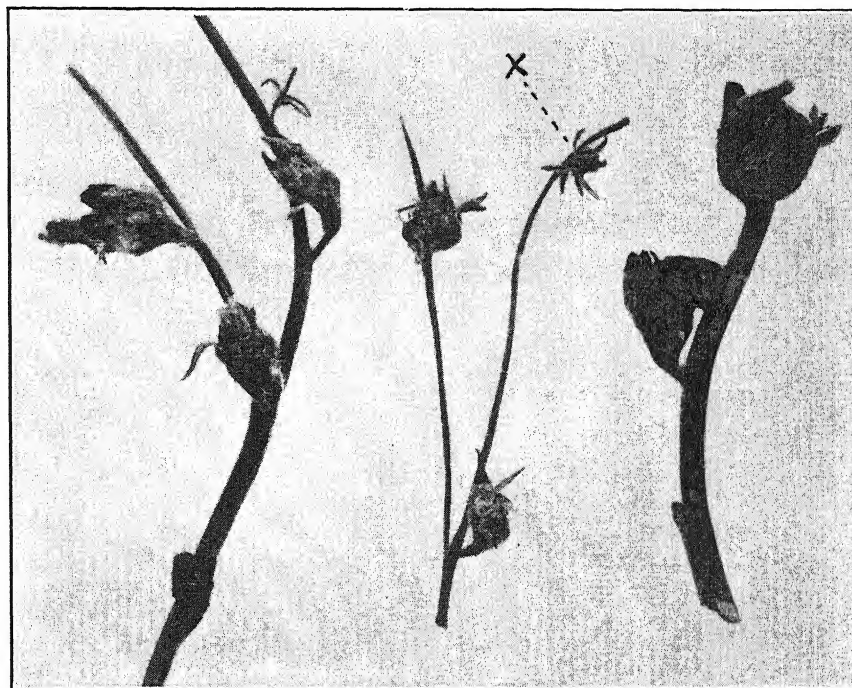


FIG. 1. Typical galls in the fruits of *Amsinckia intermedia* caused by the nematode *Ditylenchus dipsaci* var. *amsinckiae*. At *x* is shown a normal mature fruit with normal seeds. The great enlargement of the diseased fruits is manifest. $\times 1$.

LITERATURE REVIEW

There are 316 species of plants representing 40-odd families, listed as hosts of *Ditylenchus dipsaci*, in the files of the Division of Nematology, U. S. Department of Agriculture. The general symptoms, applicable to all its hosts are "more or less localized deformations of stem and leaf tissues giving rise frequently to separate or confluent galls, especially on dicotyledonous

plants" (Goodey, 7). The gall tissue or gall-like tissue is pronounced in some host plants, such as *Taraxacum* and *Hypochaeris* (2, 4), where it is found in both mesophyll and veins of the leaves; in clover (8, 9), alfalfa (3), and other legumes, in which stems are likewise swollen; in strawberry (8), where leaves, stems, and fruits are often greatly swollen and distorted; in *Narcissus* and hyacinth (7), where the swellings early occur as somewhat yellowish "spikkels" that can be felt between the thumb and fingers and that then chiefly characterize and differentiate the disease from other yellowing diseases. This symptom, however, is conspicuously lacking in some, even severely infested, host plants, *e.g.*, garlic and parsley (5, 6), in which the recognizable symptoms are dwarfing of the plant and necrosis of heavily infested tissues.

In no case has there been reported evidence of any sharp selectivity, by the nematode, of particular tissues for infestation. The infective nematodes in a favorable environment enter any succulent tissues, and, without much migration there, forthwith begin their parasitic existence and promptly reproduce their kind. In some host plants, there is a certain degree of selection of the floral organs for the late-season propagation of the overwintering generation of nematodes. This applies to *Hypochaeris radicata* L. and *Taraxacum officinale* Weber (2), in which a distortion becomes evident at the base of the inflorescence, where the nematodes are present in abundance. From this region, some of the larvae actually enter the seed capsule, where they lie adjacent to the seed, later to become wind-disseminated. But, even in these hosts, considerable multiplication of nematodes occurs within infested leaves.

THE DISTRIBUTION OF NEMATOSIS OF AMSINCKIA

The nature of the relation of any parasite to its host bears importantly on the dissemination and geographic range of the parasite and the disease induced by it. Incidence of nematosis of *Amsinckia* is of a scattered distribution. Steiner and Scott (10) report that up to September, 1934, the disease had been seen only from Winters, Woodland, Planada, and Monticello, California, loci confined to the interior valleys. The writer has since found it near Dixon, in the Sacramento Valley. The most marked infestation, however, was observed in a limited area beside the highway 15 miles south of San Jose, in the Santa Clara Valley. Here the degree of infestation every year ranges from that of a few scattered marginal plants to almost 100 per cent of those occupying the center of the area. Heavy stands of *Amsinckia* one-half mile north are entirely free, and, for a distance of 24 miles south, no further infestation was observed.

Nematosis of *Amsinckia* apparently is not widespread through the range of the host plant, but is limited to relatively small infestation foci of unknown origin. Obviously, the mode of dissemination of the parasite in this host does not facilitate its rapid and continuous spread.

THE HOST-PARASITE RELATIONSHIPS OF DITYLENCHUS DIPSACI
VAR. AMSINCKIAE

As stated by Steiner and Scott (10), infested fruits are filled with nematodes in every stage of development. Most of the adults are dead by the end of the growing season; the majority of the eggs are hatched. The contents of mature, hard, and somewhat dry infested fruits consist of a dry cottony mass of immobile larvae only partly filling the gall cavity. Within 30 minutes after moistening, the swollen mass of larval worms becomes active. The writer has estimated by aliquot-part methods that an average-size gall may contain in round numbers 180,000 nematodes. Sixty per cent of these are in the resting stage of development, mostly living; 34 per cent are younger larvae, all inactive and probably dead; and about 2 per cent are dead adults. Of 50 adults picked at random, 62 per cent were females and 38 per cent males. The random gall for this count had been 5 months on a laboratory shelf.

In nature one might expect a like reactivation of dormant larvae during moist weather. Following rain or during heavy fog, when the dried plant has absorbed considerable moisture, many of the dormant nematodes regain activity. Some of the galls are open at the top or are slightly cracked laterally, thus permitting many larvae to find their way to the surface, and thence down the stem. Numbers of them have been found among the leaf hairs along the stem, in leaf and branch-stem axils, in undeveloped flowers, and at times even within the hollowed dry stem beneath the inflorescence. During the summer, the dead plants fall to the ground. Most of the nematodes thus reach the soil on the spot, still protected during the summer and succeeding winter by the tough gall tissue.

As a rule only a small proportion of the flowers are infested. The plant, therefore, matures a large crop of normal seed. These likewise fall to the ground within the zone of infestation. Thus, for the next season, there has been provided a nearly normal yield of host-plant seed and an abundance of overwintered larvae ready to enter upon their parasitic existence. Here we have evident a highly specialized type of parasitism—one in which the population of the host plant is not endangered, yet the conditions are ideal for the propagation of a continuous population of the parasites.

Primary Infestation

In the first studies on this problem, seed of *Amsinckia intermedia* were germinated in Petri-dish moist chambers, and large numbers of infective larvae were released among them. Even after several days, no invasion of the young seedlings could be detected. Larvae were to be found on the surface of the seedlings, at the junction of the two cotyledons and in the region of the young growing point, but never within the tissues. Thus, unless some phase of the environment was distinctly adverse to infection, primary penetration of the host plant did not occur in the same manner as

with other strains of *Ditylenchus dipsaci*—as in *Hypochaeris* and *Taraxacum*, for example (2), or in clover (9) and alfalfa (3). In all of these, primary penetration takes place even in the cotyledons of the very young seedlings and, subsequently, into the interior of developing leaves and petioles.

In the early spring of 1936, the known center of heavy infestation south of San Jose was searched for young seedling plants of *Amsinckia*. On January 26, a dozen or so plants varying from 3 to 6 inches high were removed from the field and transplanted to pots in the University greenhouse at Berkeley. These grew well and, subsequently, they showed typical cases of infestation in the inflorescence. With the first collection, some of the plants were carried in moist jars to the laboratory. No evidence of invasion of the host-plant tissues was observed in stem, leaves, or bracts. In numerous cases, however, groups of infective larvae were found lying between the very young leaves surrounding the growing points. Both terminal and lateral growing points were found so infested. Figure 2, A, shows a typical case of such infestation before any sign of a developing inflorescence is evident. Figure 2, B, is a photomicrograph of a longitudinal section through a growing point a day or two older than that of figure 2, A. Sections of nematodes are evident among the leaves. None has penetrated as far as the inflorescence in the center. The method for demonstrating the presence of the nematodes in these figures and the two following was that of killing with an osmic-acid-containing fluid, followed by dehydration and clearing in clove oil, as described elsewhere by the writer (5).

In a collection made one week later, the inflorescence of the plant was evident in the terminal growing point. Examination of some of the floral buds showed nematodes penetrating individual flowers, usually basal ones. Figure 2, C, shows an entire inflorescence just emerging from the leaves at the growing point, with a group of larvae entering a single flower, not through, but between the sepals and petals. Figure 2, D, shows a longitudinal section 12 μ thick through a flower bud in a similar stage of development. The nematodes lie between the flower parts but in no case within the tissues. While no mechanical injury to flower parts is evident at this stage, there is indication that some feeding on the part of the larvae has occurred. They have definitely increased in size, and the gonad tissues have elongated and become multicellular.

Subsequently, at weekly or bi-weekly intervals throughout the period of growth of the plants, collections were made, and the mutual relations between the infesting nematodes and the developing host plant were studied. At every collection, some of the material taken directly from the plant was killed at once in Flemming's strong killing fluid or in formol-acetic-alcohol and later processed for imbedding in paraffin, and sectioned. Another portion was taken to the laboratory for examination. Figure 3 shows an entire series of collected flowers representing stages of infestation from incipency to the completely developed gall, with a normal inflorescence for

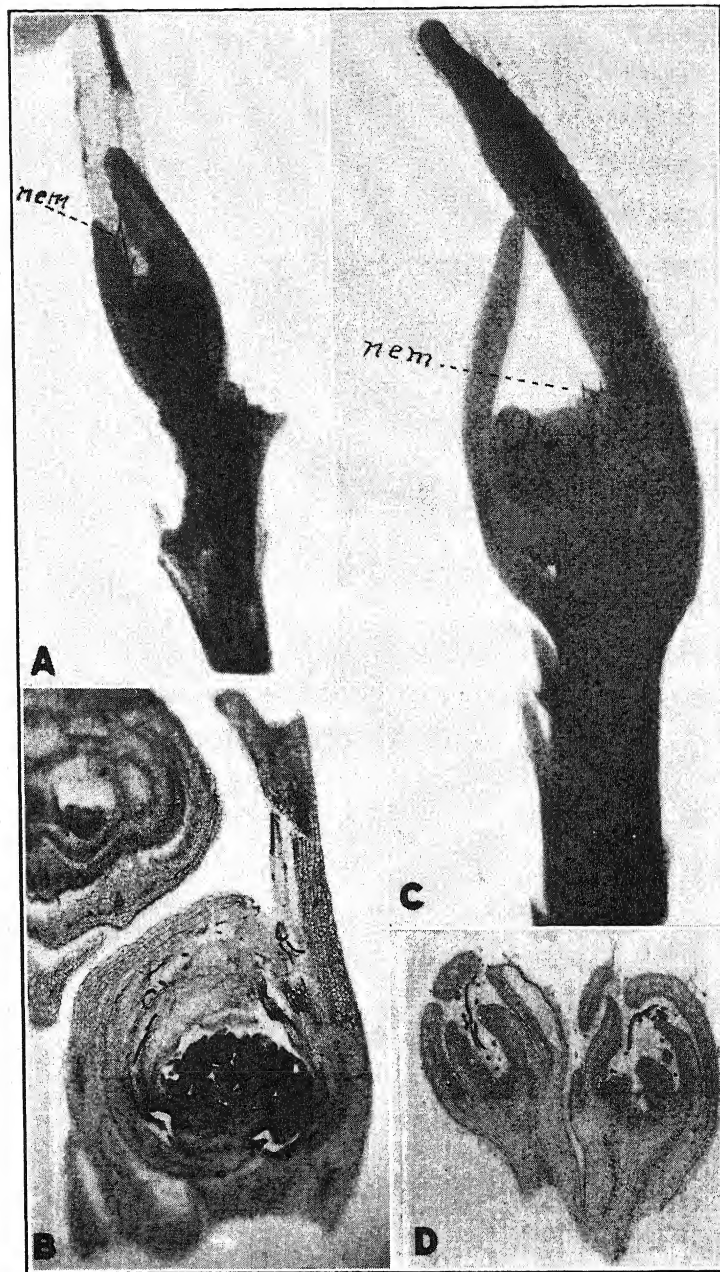


FIG. 2. A. Nematode infestation of the growing point of *Amsinckia intermedia*. The nematodes lay among the leaves surrounding the growing point, but had not penetrated any of the host tissues. They are stained black at *nem*. \times about 24. B. Longitudinal section through a growing point at a stage somewhat later than that in A. Sections of nematodes are evident among the leaves. Note the very young inflorescence, not yet reached by any of the nematodes. \times about 40. C. Single terminal inflorescence with a group of nematodes, *nem*, entering a single basal flower that was in just the right stage for penetration. \times about 24. D. Longitudinal section through two adjacent flowers in about the same stage of development as shown in C. Note the sections of nematodes beneath the petals of the flower. No development of gall tissue had occurred. \times about 40.

comparison. The sectioned material was stained in part with Flemming's triple stain, but mostly with alcoholic haematoxylin adjusted to pH 1.36 according to the method described by Craig and Wilson (1). This method gave excellent results not only in parasite-host differentiation but for the study of nuclear phenomena in host and parasite tissues as well. Figures 2, D, 4, A and B, and 5, A and B, present the sequence of events in the development of the gall.

Once the nematodes are established beneath the petals and in the cavity surrounding the very small pistil, there immediately ensues a remarkable

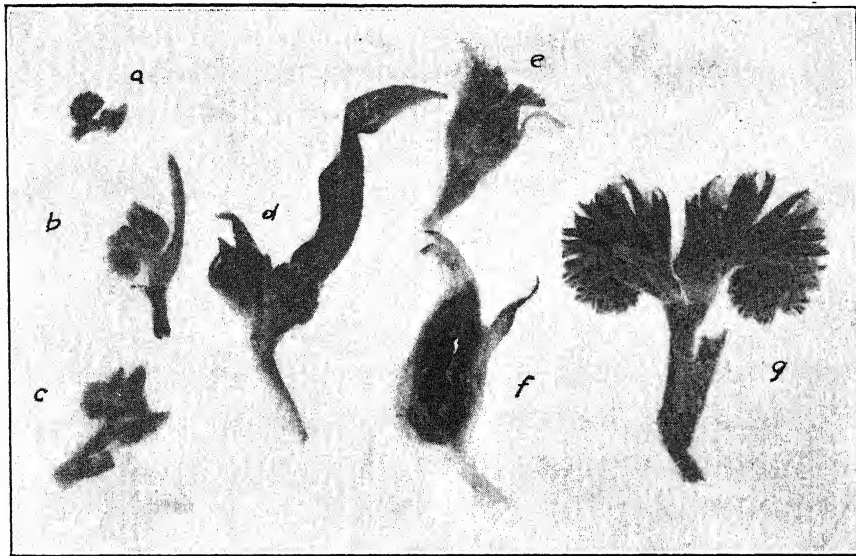


FIG. 3. Different stages of development of fruit galls in *Amsinckia intermedia*. *a*, a very young inflorescence, with a single flower infested; *b*, a similar inflorescence with all flowers, including an infested one, more advanced; *c*, a double inflorescence, with one flower in each infested; *d*, the single infested flower greatly enlarged by nematode infestation; *e*, a single infested flower, detached, approximately fully developed, as it appears at the end of the growing season; *f*, same as *e*, sectioned longitudinally, to show the interior infested region, with the slightly darkened infested tissues accentuated with a stain; *g*, a normal double terminal inflorescence, the oldest flowers having attained their maximum size. \times about $1\frac{1}{2}$.

variation from the normal course of floral development. Stimulated by the parasites, an immediate and pronounced hyperplasia of the floral organs surrounding the nematodes takes place. The highly nutritious food materials that normally go to seed production are now diverted to this abnormal growth, making for the production of a true gall tissue, and this serves as the medium upon which the nematodes feed. They develop quickly into complete sexual maturity and enter at once upon a period of intensive reproduction. The petals, normally only 4 or 5 cells thick, become many times as thick, and they grow in length also. The stamens and anthers soon lose their identity and become a part of the hyperplastic tissue. Within a few days, the nematodes are completely encased, not by

virtue of their penetration into any part of the flower or fruit tissues, but because of the growth of these parts around them. Figure 4, A, shows a single flower in this condition, with the sepals of the calyx still identifiable as such, but with the interior parts of the flower abnormally developed in size and form and completely surrounding the nematodes. These original-invading nematodes are now fully developed, and, as shown by microscopic examination, are just reaching the stage of reproduction. They are

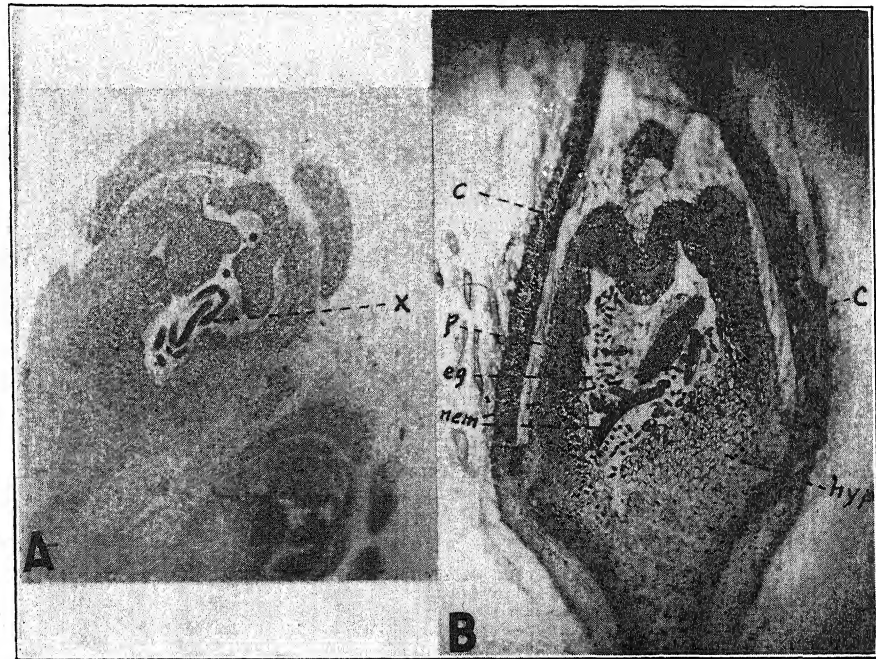


FIG. 4. A. Longitudinal section through a flower about a week older than that shown in figure 2, D. The nematodes, shown at x, (sectioned), have attained approximate adulthood, but no eggs have been deposited. The presence of the nematodes has stimulated rapid increase in host cell growth in all parts of the flower, but particularly the carpellary region. $\times 37$. B. Developing fruit gall a few days older than that shown in A. Large numbers of eggs have been deposited, and are to be seen surrounding the sections of the adult nematodes. The hyperplasia that has occurred in the host tissues has completely surrounded the mass of nematodes. C, calyx of the flower; p, abnormal petal; hyp, hyperplastic growth in the carpellary tissues of the flower; nem, adult nematodes; eg, eggs. $\times 56$.

gorged with food, and the greatly enlarged sexual organs are reflexed to nearly a quarter of their total length in extreme cases. A typical gall in this stage, about 2 mm. in diameter, contained 7 ♀ and 8 ♂ nematodes. Large numbers of eggs are produced and deposited within the cavity of the gall. The eggs, apparently without any rest period, begin the usual cell divisions; in a few days larvae appear within the cavity in abundance. A gall in the stage represented by figures 4, B, and 5, B, contained 9 ♀ and 8 ♂ adult nematodes, scores of larvae in different stages of development up to about one-half the length of the adults, and hundreds of eggs. The larvae

begin active feeding immediately and quickly grow to maturity. This second generation of mature male and female nematodes, many times the original invading population in number, start reproducing without delay. Just prior to the ripening and hardening of the plant, thousands of eggs are to be found within the cavity of the gall and deep in the smaller cavities extending into the wall.

Meanwhile, the gall has continued to enlarge greatly. Hyperplastic strands of small, sometimes irregularly attenuated cells, are evident in different regions surrounding the locule of the gall in the carpellary region at its base, in the lateral regions, where the gall tissue involves even some of the calyx cells, and even in the apical region. Sometimes uninjured cells of such strands may be recognized immediately adjacent to nematodes free in the locule. In general, however, most of the cells adjacent to the locule are collapsed and show evidence of having been the source of nutriment of the nematodes. The frequency with which cell nuclei undergo division indicates rapid growth of the hyperplastic tissue.

As the plant becomes completely mature, with drying of the stem and cessation of growth in the growing points, the eggs hatch and the larvae feeding upon the remaining nutritive material in the walls of the gall (which becomes permeated by them) attain the secondary larval stage in which they survive through the resting period. At this time adult nematodes are still to be found, as well as many newly hatched larvae. The predominating form, however, is this true resting stage, which is very uniform as to size and stage of development. Examination made in September, 1936, showed that in 3-year-old material, all the older and younger nematodes had perished leaving only this one stage capable of resuming activity.

In the surviving stage sexual differentiation was already evident, as shown by the positions of the 3-celled gonads. The alcoholic haematoxylin stain at pH 1.36, heretofore mentioned, followed by clearing in clove oil, clearly disclosed their position. This position of the gonads was recorded in a random group of 74 larvae in terms of percentage of total length from the anterior end of the nematode. In one group of 36 individuals (♀) the position averaged 54 ± 2 per cent; in the other group of 38 (♂) it averaged 77 ± 2 per cent. Thus, the sex ratio in this random lot was almost exactly 1 to 1.

These nematode-host relationship studies have clearly shown that during the growing season of the plant, the nematodes complete two full life cycles, commencing with the resting stage.

ECOLOGICAL SPECIALIZATION IN DITYLENCHUS DIPSACI

A comparison of host relations between the very highly specialized strain *Ditylenchus dipsaci* var. *amsinckiae*, on the basis of the studies herein presented, and the somewhat differently specialized strain found in *Hy-pochaeris radicata* L. reveals the extent to which ecological specialization may occur within this plant parasitic species of nematode.

Overwintering of the Nematodes

In *Hypochaeris*, the nematodes survive the winter mostly within the hardened but still green and living overwintering leaves of the plant. In *Amsinckia*, winter survival is within the hard, dry, non-living galls produced by the over-grown floral parts of the plant.

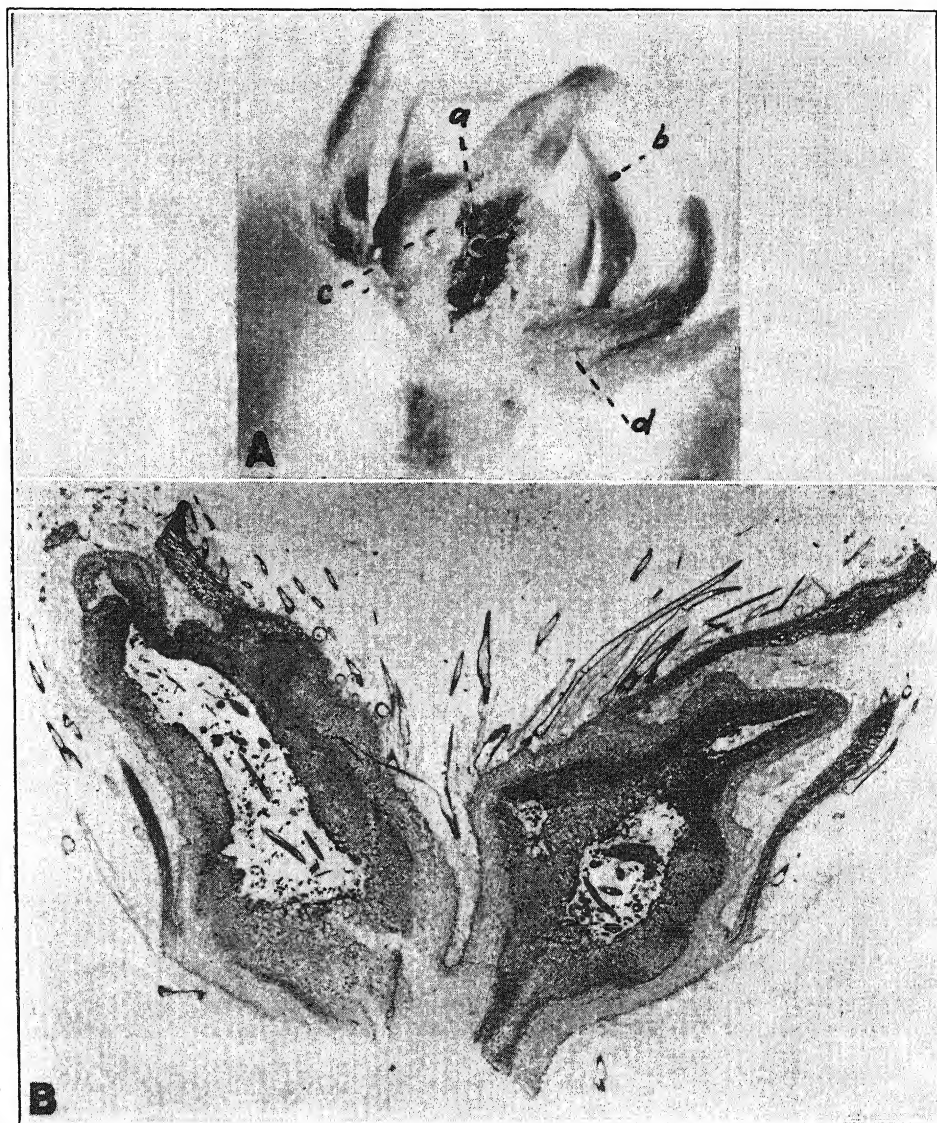


FIG. 5. A. Immature gall dissected partly open to show *a*, the mass of nematodes, adults, larvae, and eggs, in the interior; *b*, the fairly normal appearance of the calyx; *c*, the gall proper, many cells thick, completely surrounding the nematodes, the petals and other flower parts having completely lost their identity; *d*, a normal flower of about the same stage of development. B. Longitudinal section through two adjacent infested flowers, showing in the interior, sections of the original invading nematodes, now adult, and eggs and larvae of a new generation. $\times 30$.

Host-plant Infestation

Invasion of succulent leaf and floral tissues, with resulting distorted growth, is common in *Hypochoeris*. The gall-like swellings are to be found throughout the year. None whatever of this kind of invasion of tissues is to be found in *Amsinckia*.

Nematode Life Cycles

Within the gall-like leaf swellings in *Hypochoeris*, at any time in the year nematodes are to be found in all stages of development. There are no clearly differentiated generations to be detected, except those connected with the floral parts. In *Amsinckia*, there are 2 distinct generations, and only 2.

Primary Early Spring Infestation of the Floral Parts

The two plants are alike in that infective larvae are available from the over-wintering source for infestation of the floral parts as soon as they appear. In *Hypochoeris* they are washed or they actually migrate downward to the center of the plant rosette. Here the tender succulent buds appear early. The infective larvae migrate between the inflorescence bracts to their points of attachment, where they penetrate directly into the pith region at the top of the stem and beneath the plate upon which are attached the florets of the inflorescence. This normal path of entrance in *Hypochoe-*

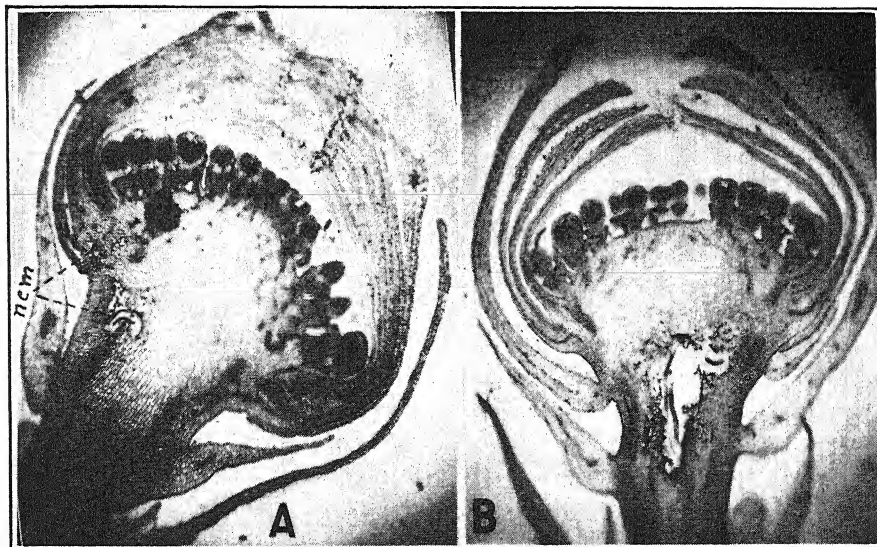


FIG. 6. A. Inflorescence of *Hypochoeris radicata* showing early stage of invasion by the nematode *Ditylenchus dipsaci*. The path of entrance is not through the individual flower, as in *Amsinckia*, but between the bracts of the inflorescence, and thence by actual penetration of the tissues, into the pith region beneath the inflorescence. *Nem*, nematodes. \times about 25. B. A later stage of infestation in *Hypochoeris radicata*. The cavity in this case is produced entirely by the mechanical activities of the nematodes, and not by any growth reaction on the part of the host plant. \times about 25.

ris is illustrated in figure 6, A. In this region of primary infection the larvae migrate freely, feed upon and destroy the cell contents, mature promptly, and reproduce. The mature adult nematode stage is shown in figure 6, B. The young migrate upward while feeding upon the parenchyma cells of the stem. This new generation of infective larvae, at just the right stage of development of the florets, penetrates the seed coat at its base and becomes established in a temporary resting stage next to the uninjured seed. With *Amsinckia*, as shown previously in this paper, infestation of the flower is by external migration among the leaves of the growing points and between the petals of the developing flower, eventually becoming localized within the flower. This is followed by hyperplasia of the floral parts completely enclosing the nematodes.

Modes of Dissemination. Herein lies one of the most striking differences between these 2 highly specialized strains of *Ditylenchus dipsaci*. In *Hypochaeris*, dissemination takes place by means of the wind-blown pappus-bearing seeds. Details of proof of this have been presented heretofore (2, 4). This results in widespread distribution of the nematosis produced by this strain. It occurs more or less uninterruptedly from Puget Sound to San Francisco Bay and in many parts of Europe and probably Asia. With *Amsinckia*, dissemination in nature depends entirely upon the survivors of the nematodes from fallen galls. The range of *Amsinckia* nematosis is correspondingly limited to small loci of infestation.

SUMMARY

The plant infesting nematode, *Ditylenchus dipsaci* var. *amsinckiae*, in its manner of producing galls in its host plant *Amsinckia intermedia* Fish. and Mey., displays a remarkable specialization in the selection of host tissues invaded. In this respect it differs strikingly from other strains of *Ditylenchus dipsaci*. Primary infestation of the plant is among the leaves surrounding the growing point, whence it enters the developing flowers. Entrance is gained not by direct tissue penetration, but by migration between the floral parts to the space adjacent to the ovary. Here their presence stimulates active hyperplastic growth of the floral parts, resulting in complete enclosure of the nematodes and the formation of a gall much larger than the normal fruit. Within this developing gall the nematodes feed and pass through two complete life cycles, eventuating in a tremendous population (as many as 40,000 in a single gall) of sexually differentiated resting-stage larvae. The firm leathery gall, fallen to the ground, serves as a protection to the nematodes until the advent of the next growing season, when they are available to initiate new infections in the seedling plants. There being no provision for widespread dissemination of the parasite, infestations of the plant are distinctly localized. The comparison is made between this manifestation of highly specialized host-parasite ecological relationship, and that occurring with the same species of nematode in *Hypochaeris radicata*. In the latter, direct tissue penetration

occurs, with development of gall-like swellings in leaves and floral parts, and in addition the seed capsules are penetrated, and the nematodes are disseminated by means of the wind-blown pappus-bearing seed. This results in widespread distribution of the *Hypochoeris* strain of the nematode.

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BROWN BLIGHT OF LETTUCE

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INTRODUCTION

This paper is a preliminary report on the brown blight of lettuce, recording investigations carried out in the Imperial Valley of California from 1922 to 1927. In the fall of 1922 the writer began the investigation of this new disease of lettuce in the Imperial Valley of California. At that time lettuce production in this section was relatively a new industry, and the disease was threatening to seriously curtail production.

Brown blight was apparently unknown until lettuce culture became important in the Imperial Valley. In a few years it increased rapidly and

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² The manuscript of this article, as prepared by Mr. Jagger, was apparently never considered by him to be a complete or conclusive treatment of the subject. It was found among his records after his death and is published essentially as originally prepared several years ago because it presents the only known detailed description of the disease, together with other observations of historical importance and technical interest. These circumstances must be kept in mind when judging the article in the light of present knowledge.

was called to the attention of State and Government pathologists by local growers in 1917 or 1918. D. G. Milbrath³ made observations on the trouble in the field and recognized it to be an undescribed disease, but did not publish on it. Since 1922 several popular accounts of the disease and its investigation have appeared in local newspapers and various agricultural publications.

COMMON NAME

When investigations were begun in 1922 various names were locally applied to the disease, but the general term "sick" or "diseased" lettuce was most frequently heard. In conferring with Imperial Valley people the name "brown blight" was hit on, and was proposed in an article published in local newspapers in February, 1924. It is now quite generally used.

DISTRIBUTION

So far as known the disease occurs only in California and Arizona. In California it is very destructive in the Imperial Valley. Occasional possibly affected plants have been found in Orange, Los Angeles, and Salinas counties. If it does occur in these counties, it is increasing much more slowly than in the Imperial Valley and is of no economic importance at present. In 1925 it was noted in Arizona in the vicinity of Yuma and Phoenix, threatening serious injury in the former section, but apparently developing less rapidly in the latter.

HOST PLANTS

Brown blight is known to attack only lettuce (*Lactuca sativa* L.). A large number of crops and many species of weeds have been observed making normal growth on soil so severely infested that 75 to 100 per cent of the comparable adjacent or preceding crop of lettuce was destroyed. Crops observed include garden pea, cowpea, cotton, cantaloupe, carrot, alfalfa, barley, grain sorghums, red table beet, and endive (*Cichorium endivia* L.).

DESCRIPTION

Seedlings are never affected until they have developed 4 or 5 leaves each, but, thereafter, plants are attacked in all stages of growth. On severely infested land, seedlings nearly always appear entirely normal until after thinning. Symptoms depend on the stage of growth at which the plants are attacked.

When attacked while small the first symptom is the appearance of small, light yellow, discolored spots in the young expanding leaves at the centers of the plants (Fig. 1). The yellow spots are very distinctive and seem to be an unmistakable symptom of brown blight. At first the spots are almost indistinguishable in bright sunlight, but can be readily seen on shading the plants. As the leaves expand the spots enlarge somewhat and the leaf areas between become sickly yellowish green. These and all subsequent leaves

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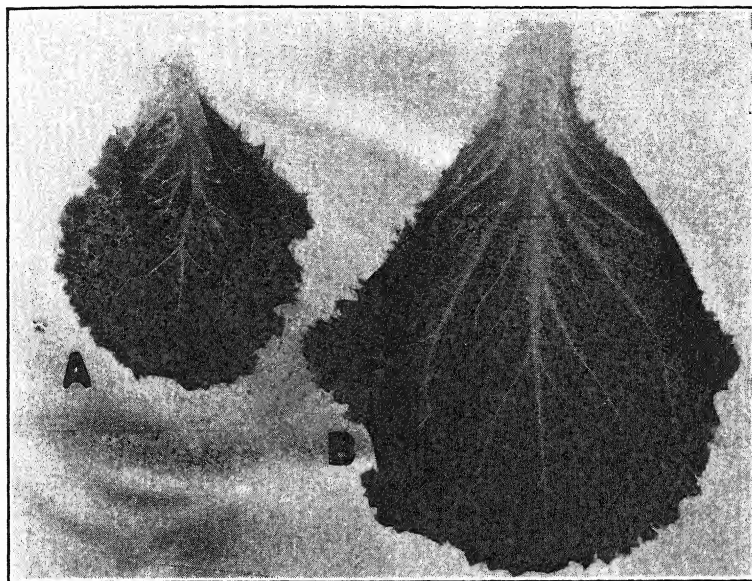


FIG. 1. A. Lettuce leaf, showing light yellow specks, the first symptom of brown blight. B. Healthy leaf.

are much reduced in size and tend to lie flat on the ground, producing small, much stunted, rosette-like, discolored plants, which are very conspicuous in the field (Fig. 2). Sometimes the older leaves at the base, fully expanded

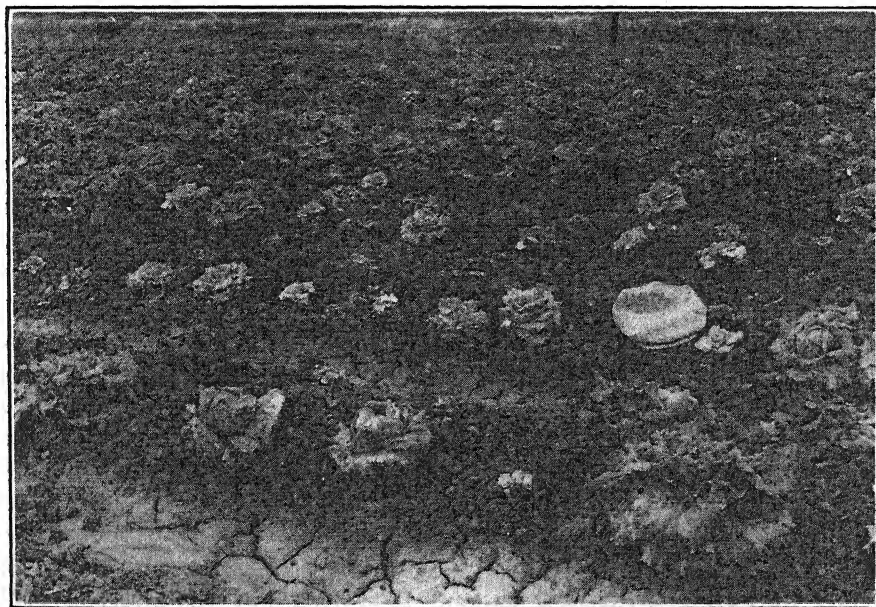


FIG. 2. Lettuce field, showing brown-blight infested area in center foreground with diseased discolored, much stunted plants in contrast with healthy plants in the lower corners and background.

when the plant was attacked, retain a healthy appearance for some time, contrasting strongly with the younger, stunted, discolored leaves above. Finally the stunted plants show a gradual browning and dying of the leaves, progressing upwards from the bases, and many plants are entirely dead before harvest.

Plants that are attacked after the heads have begun to form first show dead, brown, irregular, disconnected, more or less sunken blotches and streaks in the leaves (Fig. 3). The brown dead tissues are firm and dry

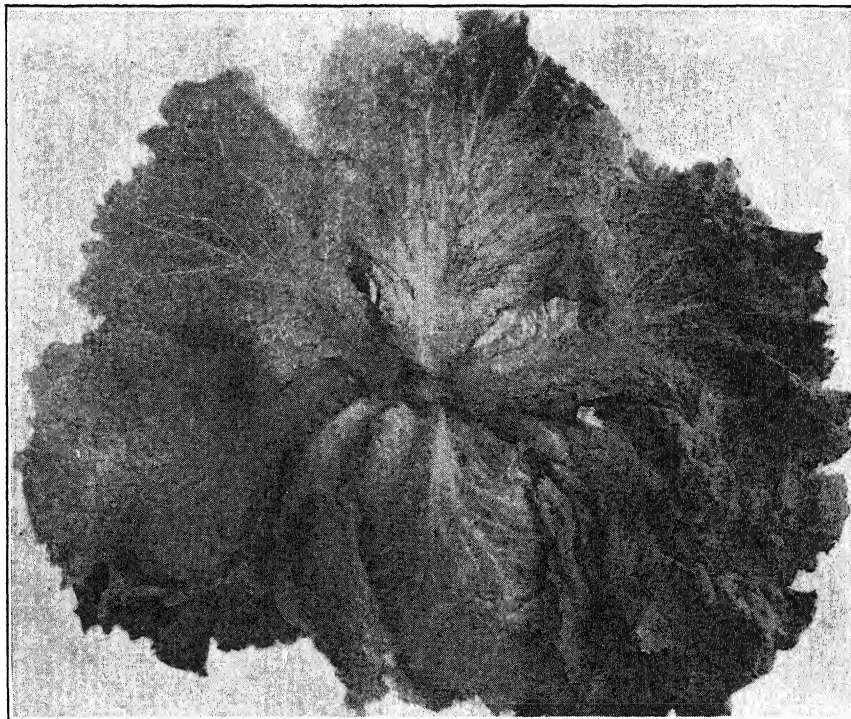


FIG. 3. Head of lettuce attacked by brown blight, with loose wrapper leaves on near side broken back, showing brown dead irregular blotches and streaks in several leaves.

unless invaded by secondary soft-rot organisms. The dead areas are variable in size, shape, and distribution. They usually are associated with the midribs and larger veins of the leaves, but may occur between veins or along the smaller veins. The dead brown streaks sometimes extend into the vascular tissues of the stem for a short distance, but are frequently confined to the leaves. In the loose wrapper leaves and the outer leaves of the head the lesions occur largely in the basal portions, but frequently extend well towards the tips in the covered leaves of the head. A large percentage of the leaves, or only an occasional leaf, may show lesions. A few of the outer leaves of the head usually show the most pronounced lesions, which become less prominent toward the center and are always absent from several layers of the smaller heart leaves at the center of the head. While these lesions are

developing, the general color of the plant gradually changes from dark green to a sickly yellowish green, the leaves and head become more or less flabby, growth all but ceases, and finally there may be a gradual browning and dying of the outer leaves.

There are, of course, all combinations of the two sets of symptoms in plants attacked in intermediate stages of growth. Additional symptoms, which seem to be of a secondary nature, frequently occur. Heads attacked when nearly mature sometimes show a dark brown, moist, slippery condition of the small heart leaves. In early stages of the disease the roots appear to be normal; but in advanced cases, when the plants are stunted and gradually dying, they show much discoloration and dying back from the tips. Affected plants are more readily injured by frost than healthy ones. Organisms, which cause soft rot or "slime," obtain a foothold in the dead brown lesions of affected plants when conditions are favorable.

In lettuce fields there may be only an occasional diseased plant, or there may be irregular areas from a few feet to several rods in diameter, within which essentially all plants are diseased. The more or less isolated infested areas where the plants are stunted and dying are very noticeable in moderately infested fields. In severe cases a high percentage of plants over whole fields may be affected. A considerable percentage of plants, destined to be affected, usually are attacked while small. An occasional crop, however, may show only a few diseased plants during the early part of the growing season and be severely attacked when nearly ready to harvest.

All plants attacked before maturity are either dead or stunted, discolored, and useless at harvest time. Occasional plants, attacked when almost ready to harvest, appear to be normal on superficial examination and are not detected in harvesting and packing. Heads showing the characteristic dead, brown, irregular streaks and blotches on removing a few outer leaves are sometimes found on the market.

BROWN BLIGHT IS SOIL-BORNE

The distribution and spread of the disease in fields is characteristic of a soil-borne trouble. Usually there is less than 1 per cent of diseased plants where lettuce is grown for the first time, but with continued cropping there is a rapid increase each succeeding year until 75 per cent or more of the plants may be diseased in the third or fourth crop of lettuce. Numerous cases have been observed where the disease was limited to a few isolated areas in a field, and in the next crop the disease was centered in the same areas, which were, however, markedly larger. Of two fields separated only by a ditch or a fence, one often shows a high percentage of diseased plants, usually where previous crops of lettuce have been grown, and the other, only a few feet distant, an unimportant percentage.

In numerous experiments pots and boxes of soil from severely diseased fields or areas produced lettuce that showed nearly 100 per cent characteristic brown blight, while comparable pots and boxes containing soil from

healthy fields or areas or from uninfested regions produced disease-free lettuce. The disease developed fully as well in the coastal climate at Chula Vista, San Diego County, Calif., as in the decidedly different Imperial Valley, and all experiments of this nature were carried out there. In most of the experiments plants were grown in 6-inch clay pots, usually 4 plants in a pot. Plants were somewhat crowded and undersized in later stages of growth, but grew sufficiently well to readily show brown-blight symptoms. When plants became an abnormally yellowish color, indicating lack of nutrients in the limited soil, they were quickly brought back to normal by watering for a few days with water containing 0.1 per cent of NH_4NO_3 and 0.05 per cent of $\text{K}_2\text{HP}_2\text{O}_5$. There were never any indications of the disease being carried from diseased to healthy pots by insects or by soil splashed in watering, although diseased and healthy pots were adjacent in many cases.

NOT ALKALI POISONING

It has been repeatedly suggested that the trouble might be because of an excess of alkali salts in the soil, a supposition based apparently on the facts that brown blight, like alkali poisoning, occurs first in isolated areas that increase in size from year to year, and that alkali occurs in all known brown-blight-infested regions. Since germinating seed and small seedlings never are affected by brown blight, no other crops or weeds seem to be susceptible, and since the usual symptoms of alkali poisoning are lacking, alkali poisoning seems precluded. In order to test definitely the alkali-poisoning theory, sufficient distilled water was leached through pots of infested soil to reduce the content of soluble salts to a very small percentage of the original, as determined by the electrical-bridge method (1). There was fully as high a percentage of brown-blight-diseased plants in the leached pots as in untreated check pots.

SOIL STERILIZATION DESTROYS CAUSAL AGENT

The close association of the disease with the soil suggests an organism parasitic on the roots. In order to throw further light on this possibility pots were filled with infested soil; a portion was sterilized by steaming 1 hour under 10 to 15 pounds' pressure; part was sterilized by drenching with 40 per cent formaldehyde, diluted 1 part to 20 parts of water; part was given no treatment. Lettuce was grown in all under like conditions. The combined results from 3 experiments were 58 healthy and 0 diseased plants in 15 steam-sterilized pots; 60 healthy and 0 diseased plants in 15 formaldehyde-sterilized pots; and 2 healthy and 58 brown-blight-diseased plants in 15 untreated check pots.

ORGANISMS

Attempts were made to isolate an organism from the brown dead lesions of older plants with no indications of success. Later, as a result of the soil experiments, the search was transferred to the roots. As already stated roots appear to be entirely normal in early stages of the disease but micro-

scopic and cultural examination shows a considerable number of fungi associated with them. A large percentage of the roots of lettuce and many other crops and weeds in Imperial Valley are heavily invaded by an organism that is indistinguishable from the mycorrhizal fungus studied by Jones (5). This is fully as abundant in healthy as in diseased plants and apparently has no connection with brown blight. Several species of *Pythium*-like fungi were isolated from the roots of diseased plants and tested as possible causes of the disease, although studies to the present time indicate that the same species are associated also with the roots of healthy plants.

Finally, a fungus, which seems to be *Asterocystis radialis* de Wildeman, has been repeatedly found in abundance in the epidermal cells and root hairs of diseased plants, and has been found only rarely and in limited amounts in healthy plants. This fungus is widespread in Europe, where it has been recorded in the roots of many species of plants by de Wildeman (8), Marchal (6), Ducomet (2), Fron and Gaillat (3), and others. In most species the fungus seemed to cause no appreciable injury to the hosts. Diseases of flax, oats, and grasses, however, are attributed to it in the last three references, although entirely conclusive proof of a causal relation was obtained in no case. As in the European investigations, inability to grow the fungus in pure culture has made it difficult to determine whether it causes disease in lettuce. At present, *Asterocystis radialis* can be mentioned only as a possible cause of brown blight of lettuce.

SYMPTOMS SUGGEST A MOSAIC

Brown-blight symptoms are suggestive of a transmissible mosaic disease, although the common mosaic disease of lettuce (4) is very distinct from brown blight. Unsuccessful attempts were made to transmit brown blight by the usual experimental methods of transferring insects and that of injecting juice from diseased plants, but that line of investigation was abandoned on discovering the soil relations of the disease. Further studies are now indicated, since brown blight is strikingly similar in many respects to the disease of wheat and rye, shown by McKinney (7) to be a transmissible virosis, the causal agent of which is associated with the soil. There are no indications that the two diseases are identical, but they might well be of similar nature.

CONTROL

Crop Rotation

On account of lettuce culture being a comparatively new industry in infested regions, only limited data on the effect of crop rotation have been obtainable. It has, however, been conclusively demonstrated by the experiences of several growers that growing alfalfa or other crops besides lettuce for 3 or 4 years causes little or no reduction of infestation. In fact, general observations have in several cases suggested an increase in soil infestation during 1 to 4 years of crops other than lettuce, but the increase was much slower than with lettuce.

Avoiding Infested Land

In the past, sufficient acreage suited to lettuce has been available, so that serious losses have been largely avoided by constantly shifting to land where lettuce has never before been grown. In the Imperial Valley diseased plants can be found in nearly all fields, but there is usually less than 1 per cent where lettuce is being grown for the first time. Occasionally, there is sufficient disease in first-year lettuce to cause appreciable reductions in yield. Second-year lettuce usually makes a satisfactory crop; but, in general, there is a higher percentage of brown-blighted plants, and cases of economic losses are more numerous. Usually, third-year lettuce is so seriously injured that it does not make a profitable crop, although occasional third-year and even fourth-year crops show only limited disease. In the Imperial Valley it has become customary to grow two crops of lettuce on land, and then to pass on to land where lettuce has never been grown before.

Careful counts were made in a first-year lettuce field of 10 acres that showed more brown blight than usual, and the next season similar counts were made in second-year lettuce on the same field to obtain information on rate of increase. A total of 4,000 plants in the first-year crop showed 3.7 per cent of brown blight, and the same number in the second-year crop showed 20.3 per cent of brown blight. In another case, which is perhaps more typical, there were only 2 (0.05 per cent) diseased plants among 4,000 in first-year lettuce and 101 (2.5 per cent) among 4,000 in the second-year crop. In a second-year field of 20 acres, estimates as well as yields indicated that 75 per cent of the plants were affected with brown blight. Many third-year fields have been observed where counts and estimates have indicated from 15 per cent to as high as 90 per cent of diseased plants.

These data, as well as general observations, indicate that on land where a crop shows over 1 per cent of disease it is in general not advisable to grow another crop of lettuce. So far as is known at present it is not safe to grow lettuce on land where this crop has been grown in previous years and followed by other crops for several years. Occasionally crops are injured by brown blight in spite of every precaution to avoid infested land, since land that has never grown lettuce is sometimes infested, and since the previous cropping may not be accurately known.

RESISTANT VARIETIES

In 1923 over 100 varieties of lettuce were grown on infested land. As the number of plants was limited and the field showed areas of light infestation the results were not conclusive. Most of the varieties showed some brown blight, but the two varieties, Big Boston and Chavigne, were disease-free. Several hundred plants of these two varieties were grown on severely infested soil in 1924 and again in 1925 and made entirely normal growth, with no indications of brown blight, whereas check plants of the New York variety showed a high percentage of disease. It thus seems conclusively demonstrated that both varieties are highly resistant, if not entirely im-

mune. Several hundred second-generation hybrids from a cross between Chavigne and New York were grown on severely infested soil in 1924, and in 1925 gave healthy and diseased plants in ratios of approximately 3 to 1, thus indicating that resistance to brown blight probably behaves as a Mendelian dominant character. The obtaining of brown-blight-resistant strains of the New York type by selection from the hybrids seems possible, although progress in that direction is now overshadowed by the resistant selections from the New York variety.

The brown-blight-resistant strains, obtained by selecting within the variety New York, seem to show no resistance to the obscure and so far unimportant pathological condition designated as "big vein." Big Boston and Chavigne, however, seem to be resistant to or immune from both diseases. Commercially, these varieties are of little or no value in infested regions, but hybridizing with New York offers possibilities of obtaining New York types that are resistant to both brown blight and "big vein."

Resistant Strains of Variety New York

In 1924 a field of badly diseased lettuce was found where many of the plants showed marked indications of resistance (Fig. 4). One hundred

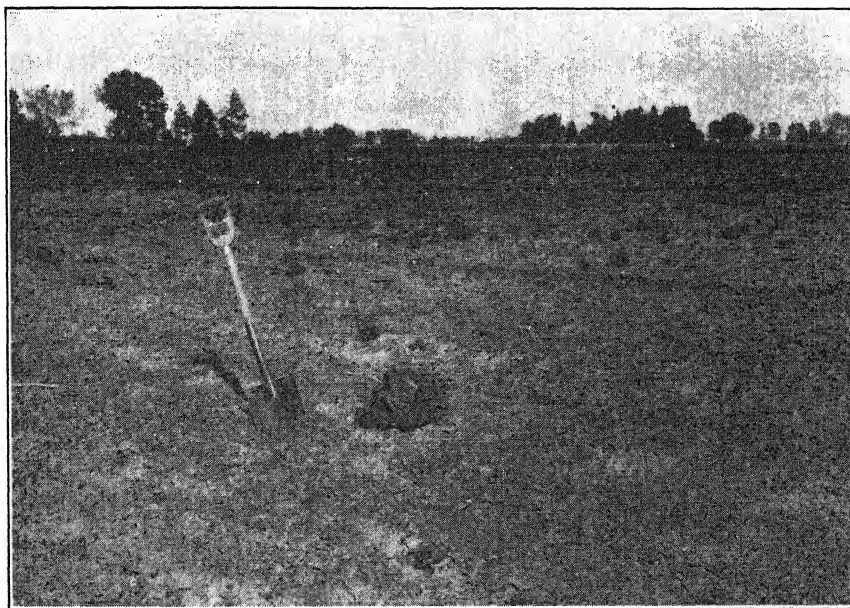


FIG. 4. Parent plant of brown-blight-resistant lettuce, New York Imperial No. 2, as found in severely diseased field with all surrounding plants diseased or dead, Imperial Valley, 1924.

promising plants were selected and seeded. As lettuce is largely self-pollinated, no precautions against cross-pollination were necessary. From each of the 100 lots of seed 25 to 100 plants were grown on infested soil in 1925.

From the time brown blight commenced to develop it was evident throughout the season that a surprisingly large number of the selected strains possessed a pronounced degree of resistance. Commercial seed planted as a check showed a high percentage of brown blight in all parts of the trial area, 85.5 per cent of a total of 2,362 check plants being diseased. In 55 of the selected strains there were no diseased plants throughout the season. It was hoped that some of these might prove to be entirely immune, but, as recorded below, all strains so far tested on a large scale on heavily infested soil have developed a small percentage of disease. Of the remaining 45 strains nearly all showed indications of resistance.

The field where the above selections were made apparently was planted with different commercial seed from that commonly used, and finding the field seems to have been a matter of good fortune, for it resulted in resistant strains being isolated and established almost immediately. The lettuce in this field seemed typical of the variety New York, although there were more sports and off-type plants than in most fields. Search for resistant plants had been made in many fields without finding anything of promise until this field was visited. At the time of making selections in this field, 35 less promising plants were selected and seeded in 3 other fields where there was the usual absence of plants showing definite indications of resistance. When these 35 lots of seed were tested in 1925, there was no indication of resistance in 30, while the other 5 lots, though somewhat resistant, were otherwise worthless.

Most of the selections made in the promising field were of the New York

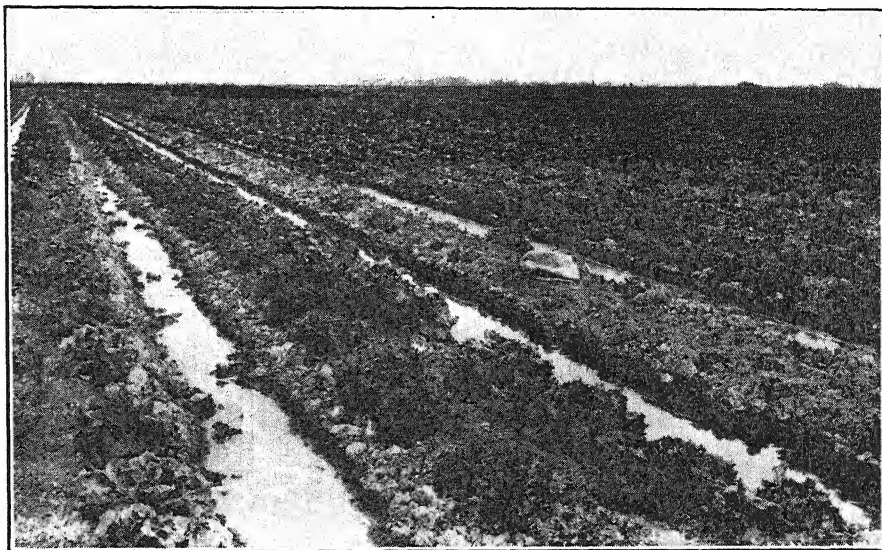


FIG. 5. Experimental planting, 1926. In center, a double row from non-resistant commercial seed with over 90 per cent of the plants diseased and dying. On each side, a 2-acre block of selected resistant strains numbers 2 and 3 with less than 0.5 per cent plants diseased by brown blight.

type, although some sports and off-types were included. In the 1925 progenies some strains were extremely variable; others were unusually uniform. Of the highly resistant, reasonably uniform strains, several that differed appreciably from each other in minor characters were very similar to the New York variety. As soon as the 1925 plantings reached a stage where the commercial value of the different strains could be judged, the 3 most promising were chosen for growing sufficient seed for commercial trial in 1926. The remaining seed of these strains, obtained from the 3 parent plants selected and seeded in 1924, was then planted in a favorable locality, and several pounds of seed of each strain were harvested in the autumn of 1925.

In 1926, 2 acres of each of the 3 resistant strains were grown in a severely infested experimental field, and smaller areas in several commercial fields where there was only a limited amount of disease. All 3 strains showed a high degree of resistance (Fig. 5). All developed a small and commercially unimportant percentage of brown blight on severely infested soil, and also a small percentage of the obscure pathological condition designated as "big

TABLE 1.—Percentages of diseased plants in trial plantings of selected brown-blight-resistant strains of the New York variety of lettuce and of commercial seed of the same variety in adjacent check rows, Imperial Valley, California, 1926

Row No.	Varieties and strains	Counts made 5 to 6 weeks before harvest		Counts made a few days before harvest	
		Total plants	Brown-blight-diseased plants	Total plants	Brown-blight-diseased plants
		Number	Per cent	Number	Per cent
	<i>Severely diseased experimental field</i>				
16	Resistant strain No. 1	1263	0.08	1251	0.08
17	Commercial	1022	69.77	916	92.47
42	Commercial	1063	78.27	901	90.46
43	Resistant strain No. 2	1182	0.17	1157	0.35
82	Resistant strain No. 2	1210	0.25	1176	0.33
83	Commercial	1133	76.10	912	94.30
123	Resistant strain No. 3	1090	0.55	1169	0.19
124	Commercial	1311	69.03	1110	92.07
125	Resistant strain No. 1	1191	0.25	1183	0.25
	<i>A commercial field—2nd-year lettuce</i>				
85	Resistant strain No. 3			1350	0.07
86	Commercial			1427	3.64
93	Resistant strain No. 2			1256	0.00
94	Commercial			1433	3.63
97	Resistant strain No. 1			1411	0.00
98	Commercial			836	5.03
	<i>Another commercial field—2nd-year lettuce</i>				
61	Commercial	958	5.50		
62	Resistant strain No. 1	1092	0.00		
71	Commercial	1040	0.58		
72	Resistant strain No. 2	1081	0.00		
81	Commercial	958	0.63		
82	Resistant strain No. 3	867	0.00		

vein." The strains were very uniform in type but quite distinct from each other. Plants of all three types occur in nearly all commercial fields, but in the better commercial fields most of the plants are very similar to those of Strain No. 2. Strains 1 and 3 are of very doubtful commercial value, since they frequently fail to head as well as plants from commercial seed. Strain No. 2 gives promise of being a very satisfactory commercial lettuce for the Imperial Valley on either infested or disease-free soil. It has not been tested in other sections. The following table (Table 1) gives percentages of diseased plants in the 3 strains and in check plantings of high grade non-resistant commercial seed.

The promising strain, No. 2, has been given the name Imperial No. 2. The demand for seed of this strain was becoming so great that it seemed necessary to turn the growing of seed into commercial channels. Stock seed, identical with that used in the 1926 trials, was turned over to two competing seed growers, who supply a considerable proportion of the seed used in Imperial Valley for growing seed crops during the summer of 1926.

SUMMARY

Brown blight, a new disease of lettuce, is causing increasing losses in the Imperial Valley of California and in parts of Arizona. It has been shown to be soil-borne. A root parasite is suspected as the cause, although the striking similarity of brown blight to the soil-borne mosaic disease of wheat suggests the possibility of a similar nature. Crop rotation gives little promise of control. Heretofore, losses have been largely avoided by growing usually two crops of lettuce then shifting to land where lettuce has never been grown. Certain varieties are highly resistant or entirely immune, but are commercially useless in the infested regions. Through selection from the almost exclusively grown, very susceptible variety New York, a highly resistant strain has been obtained which is coming into commercial use under the name Imperial No. 2.

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DIURNAL CYCLE OF SPORE MATURATION IN CERTAIN POWDERY MILDEWS¹

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INTRODUCTION

Massee's⁴ observations of *Sphaerotheca humuli* on vegetable marrow indicated that dissemination of conidia occurs principally at night. Hammarlund⁵ made extensive and detailed studies of the process of spore maturation in several powdery mildews. He reported that the number of conidia formed per day per conidiophore of *Erysiphe communis* (*E. polygoni*) varied from 1 to 6, and that the conidia were forcibly discharged a distance of 10 to 20 conidial lengths, but he reported no diurnal periodicity. Active discharge also was found by him in *Sphaerotheca pannosa* and other conidial chain forming Erysiphaceae. Yarwood⁶ reported that *E. polygoni* on clover showed a marked diurnal cycle in several aspects of its development, and that each conidiophore formed 1 conidium per day, which was passively liberated about midday.

In the conidial chain-forming powdery mildews *Erysiphe cichoracearum* and *Sphaerotheca* spp. Blumer⁷ has reported that the basal cell is the conidial-mother cell (generative cell). Foëx's⁸ drawings of the evolution of the conidiophore of *Sphaerotheca humuli*, indicate division of the basal cell and also division of the cell just above it. In the non-chain-forming powdery mildew *E. polygoni*, the generative cell is separated from the sporiferous hypha by a stipe cell.^{6,7}

MATERIALS AND METHODS

In this paper are reported studies on the diurnal cycle of morphological development of the conidiophores of *Erysiphe cichoracearum* DC. from *Helianthus annuus* L., from *Cucumis sativus* L. and from *Aster* sp.; of *Podosphaera leucotricha* (E. and E.) Salm. from *Pyrus malus* L.; of *Sphaerotheca pannosa* (Wallr.) Lévl. from *Rosa* sp.; of *Erysiphe polygoni* DC.

¹ The advice and assistance of Dr. C. E. Yarwood, Division of Plant Pathology, University of California, Berkeley, California, in the preparation of this paper is gratefully acknowledged.

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³ The assistance of non-technical employees of the Federal Works Progress Administration is acknowledged.

⁴ Massee, G. E. On the origin of parasitism in fungi. Roy. Soc. [London] Phil. Trans. 197B: 7-24. 1905.

⁵ Hammarlund, C. Zur Genetik, Biologie, und Physiologie einiger Erysiphaceen. Hereditas 6: 1-126. 1925.

⁶ Yarwood, C. E. The diurnal cycle of the powdery mildew *Erysiphe polygoni*. Jour. Agr. Res. [U. S.] 52: 645-657. 1936.

⁷ Blumer, S. Die Erysiphaceen Mitteleuropas mit besonderer Berücksichtigung der Schweiz. 483 pp. Gebr. Fretz A. G. Zürich. 1933.

⁸ Foëx, E. E. Evolution du conidiophore de *Sphaerotheca humuli*. Bull. Soc. Mycol. France 29: 251-252. 1913.

from *Phaseolus vulgaris* L., and of *Oidium euonymi-japonici* (Arcang.) Sacc. from *Euonymus japonicus* L.f.

Leaves recently infected with powdery mildew were gathered every 2 hours over 24-hour periods at Berkeley, California. By folding the leaf, or cutting a narrow strip of the lamina, a row of erect conidiophores was obtained that was examined microscopically with a high power objective. For sunflower, cucumber, bean, and apple powdery mildews material was obtained from greenhouse plants; material for other mildews was from outdoors.

The conidiophores studied were of 2 types reported by Blumer.⁹ The *E. polygoni* (*E. communis* Wallr.) type are relatively simple in structure and consist of a basal cell, a generative cell, and one or two maturing conidia, depending on the time of day observed. This type of conidiophore will be referred to herein as a non-chain-forming type. Conidiophores of the *E. cichoracearum* type consist of a more or less cylindrical stipe or basal cell, 1 to 3 cylindrical cells above the basal cell, and a chain of from 2 to 8 or more swollen conidia. This type of conidiophore will be referred to herein as a conidial chain-forming type (Fig. 1). Because of the vari-

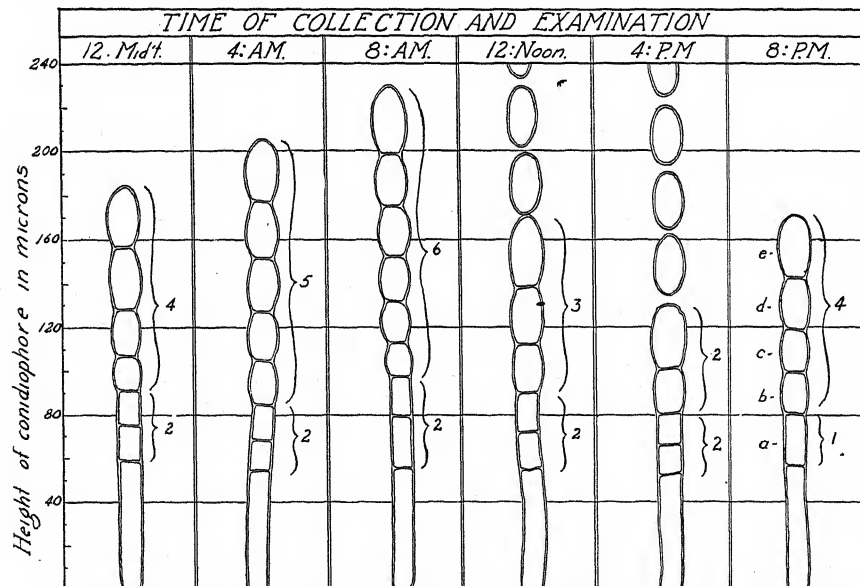


FIG. 1. A diagram of the diurnal cycle of maturation and abstriction of conidia in the cucumber powdery mildew, *Erysiphe cichoracearum*. The line between swollen and cylindrical cells was drawn where there was the greatest difference between their respective shapes.

able number of cylindrical cells above the basal cell, and the variable number of swollen cells above the cylindrical cells, a numerical method of designating the various stages in the development of the conidiophore was necessary for recording the observations. The basal cell was not considered in the final numerical records because it was relatively constant in length

⁹ See footnote 7.

throughout the diurnal cycle. The cells distal to the basal cell were classified according to a binumerical scheme: the first member of the binumerical, e.g. 3+2 (Fig. 1 at 12: Noon), refers to the number of swollen cells in a chain of conidia, and the second member refers to the number of cylindrical cells above the basal cell. The number of swollen cells of a conidiophore may amount to 10 or more, e.g. *S. pannosa* on rose, but the number of cylindrical cells above the basal cell is rarely more than 3. Abstricted but still adhering spores (Fig. 1 at 12: Noon) are disregarded.

The above observations were made during the summer months as follows: *Erysiphe cichoracearum* on sunflower, *Sphaerotheca pannosa* on rose, *E. cichoracearum* on aster, during the middle of July, 1937; and *E. cichoracearum* on cucumber, *S. pannosa* on rose, *Podosphaera leucotricha* on apple, *E. polygoni* on bean and *Oidium euonymi-japonici* on *Euonymus* in June and July, 1938.

To supplement the information secured by the direct observation of living conidiophores, the diurnal cycle of conidiophore development was followed by determining the number of spores liberated from infected leaves at periodic intervals, and by observations of the nuclei in stained conidiophores.

To determine spore liberation, young naturally infected sunflower leaves, excised and kept alive on 5 per cent sucrose solution, in Petri dishes and in a well lighted room, were removed periodically and snapped vigorously into a pint can, at the bottom of which a clean slide had been placed. It is believed that in the main, only conidia that have been abstricted will be dislodged by snapping. The number of conidia thus caught was determined microscopically.

To determine the nuclear condition in the conidiophore, primary leaves of sunflower infected with *Erysiphe cichoracearum* were collected periodically from greenhouse-grown plants in December, 1938, and cut into narrow strips bearing rows of erect conidiophores. These conidiophore-bearing strips were then fixed in formalin-alcohol-acetic acid, stained with acid fuchsin in water and examined microscopically.

RESULTS

Diurnal Cycle of Sporulation as Shown by Microscopic Examination

Examination of the stages of conidiophore development at 2-hour intervals during 24-hour periods reveals a definite diurnal cycle of maturation and abstriction of conidia in the chain-forming powdery mildews, *E. cichoracearum* (Fig. 1, 2, Table 1), *Sphaerotheca pannosa* (Fig. 2, Table 2), and *Podosphaera leucotricha* (Fig. 2). Diurnal cycles of maturation and abstriction of conidia also were observed in the non-chain-forming mildews, *Erysiphe polygoni* (Table 3) and *Oidium euonymi-japonici* (Table 4). With reference to *E. cichoracearum* on cucumber (Fig. 1, Table 1) the modal binumerical type (heavy type) was 6+2 at 6 to 8 a.m.; by 2 to 4 p.m. it had decreased to 2+2, due to the rapid abstriction of conidia during

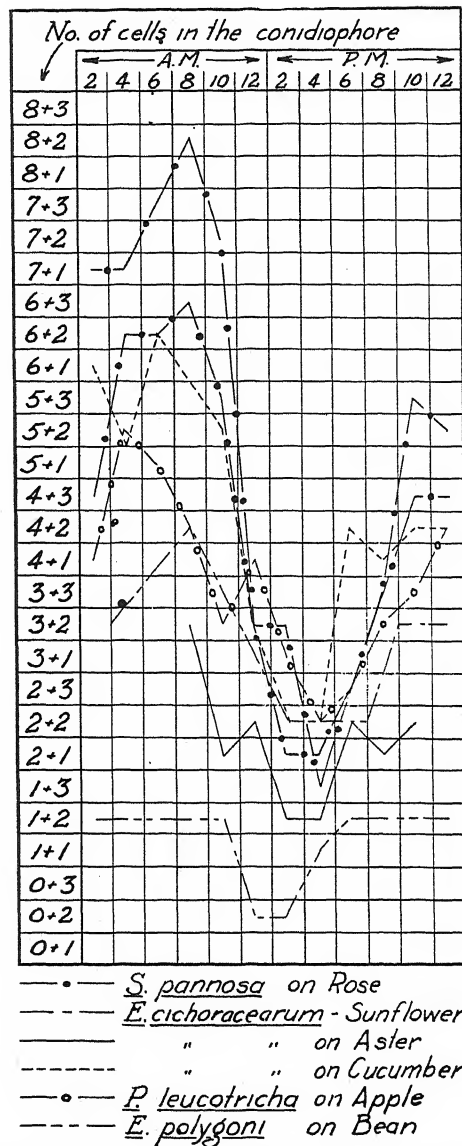


FIG. 2. A graphical summary of the diurnal cycles observed for conidial chain-forming powdery mildews and a comparison between the diurnal cycles of chain-forming powdery mildews and a non-chain former, *Erysiphe polygoni*. *Oidium Euonymi-japonici* is not represented but is very similar to *E. polygoni* in sporulation diurnal cycle. Curves are plotted from the modal binumerical type for each mildew at each period of observation. There is variation among the chain-formers, but none approach the simplicity and regularity of the cycle of the non-chain former, *E. polygoni*.

the elapsed time. From 4 p.m. to 6 a.m. the modal binumerical type rose again to 6 + 2 due to the formation of the succeeding crop of conidia. At the low phase of the cycle, e.g., 2 p.m. to 4 p.m. (Fig. 1 and Table 1) the modal binumerical type was clearly defined, while at the high phase of the

cycle, e.g., 4 a.m. to 10 a.m., the modal type was less clearly defined owing to the presence of young conidiophores that were sporulating for the first time, and old conidiophores whose activity was waning. Hammarlund¹⁰ reports the life of a conidiophore as 4 to 6 days for a number of Erysiphaceae. Similar diurnal cycles were observed for powdery mildews of rose, aster, sunflower, and apple (Fig. 2, Table 2).

TABLE 1.—Diurnal cycle of maturation and abstriction of conidia of the cucumber powdery mildew, *Erysiphe cichoracearum*. Each number in the body of the table represents the number of conidiophores of the binumerical type indicated that were observed at the time indicated. The modal binumerical type for each period of observation is printed in heavy type. The diurnal cycle of conidiophore development is graphically illustrated by the vertical fluctuation in bold-face numbers from 2 a.m. to 12 p.m.

No. of cells in conidiophore exclusive of basal cell	Diurnal cycle of maturation and abstriction of conidia											
	a.m.						p.m.					
	2	4	6	8	10	12	2	4	6	8	10	12
8+3												
8+2			4									
8+1			5	1								
7+3		1	4	1								
7+2	1	1	10	5	1							1
7+1	1	3	4	5	1						2	2
6+3	6	2	7	5	1				1			
6+2	14	14	18	15	9						2	19
6+1	23	18	4	4	4					4	14	20
5+3	12	9	14	4	8	2					1	9
5+2	22	23	4	6	23	2			6		16	21
5+1	14	23	2		10	2			8	14	15	6
4+3	14	7	3	4	20	2			5	8	3	3
4+2	9	14	6	12	19	14			21	17	17	25
4+1	5	10	6	5	12	2		1	12	26	13	13
3+3		1			8	4			11	5		1
3+2	1	3	1		1	24	8	5	7	18	5	3
3+1		3					5	11	2	6	1	
2+3	1			2	3	2	5	7	4	5	1	
2+2	2		3	2	7		24	27	1	5		2
2+1	5	1	1	1		1	4	6	1	1	3	1
1+3							14	5		1		
1+2							1	8				
1+1						1						

Conidiophores of the *Erysiphe polygoni* type characteristically form no spore chains and have a diurnal cycle of sporulation that may be represented by a more flattened curve (Tables 3 and 4). Only one spore, on the average, was abstricted during a 24-hour period as compared to 4 to 6 or more in the conidial-chain-forming powdery mildews observed. The cycle of sporulation found for *E. polygoni* on beans corresponded closely to that found for the same conidiophore type on *Euonymus*, and with that observed by Yarwood¹¹ for clover mildew, though Yarwood's data were presented in a different form. Abstriction of conidia (Tables 3 and 4) started about 10 a.m. and was completed by 2 p.m., or soon thereafter. Active discharge of conidia, as described by Hammarlund,¹⁰ was not observed in

¹⁰ See footnote 5.

¹¹ See footnote 6.

TABLE 2.—*Diurnal cycle of maturation and abstriction of conidia of the powdery mildew of rose, Sphaerotheca pannosa. Each number represents the percentage of the binumerical type indicated, observed at each period of collection and observation. The highest number for each observation period is printed in bold-face type. The diurnal cycle is illustrated by the vertical fluctuation in bold-face numbers from 2 a.m. to 12 p.m.*

No. of cells in conidiophore exclusive of basal cell	Diurnal cycle of maturation and abstriction of conidia											
	← a.m. →						← p.m. →					
	2	4	6	8	10	12	2	4	6	8	10	12
8+3												
8+2												
8+1		1.5	11.3									
7+3		2.9	5.6	8.3								
7+2	1.3	4.4	11.3									
7+1	2.6	13.2	11.3		2.3							3.6
6+3	2.6	7.4	11.3	22.2	4.6						4.2	
6+2	11.8	19.1	14.1	2.8	15.9						8.3	7.3
6+1	15.8	2.9	2.8	13.9	13.7					3.1	2.1	18.2
5+3	7.9	10.3	14.1	8.3	25.0						29.2	5.5
5+2	7.9	1.5	8.5	11.1	20.5					2.0	18.8	30.9
5+1	9.2	2.9		2.8	2.3	2.0			1.9	14.3	4.2	7.3
4+3	18.4	7.4	4.2	5.6	6.8				1.9	17.4	16.7	7.3
4+2	7.9	5.9	4.2	8.3	4.6	2.0			11.4	18.4	4.2	12.7
4+1	9.2	4.4			2.3	17.7			10.5	14.3	2.1	5.5
3+3	2.6	1.5			2.3	5.9			17.1	21.4	4.2	1.8
3+2	2.6	4.4				37.2	1.2	1.2	18.1	4.1	2.1	
3+1		5.9		2.6		13.8	8.4	8.6	7.6	1.0		
2+3		1.5		2.8		13.8	1.2	1.2	19.1	3.1		
2+2		2.9		2.6		3.9	18.1	4.8	7.6		2.1	
2+1				2.8			20.5	30.9	1.9	1.0	2.1	
1+3						3.9	19.3	11.1	1.9			
1+2							15.7	23.5				
1+1							15.7	18.3				

these studies, and abstricted conidia were commonly observed adhering to the conidiophore.

TABLE 3.—*Diurnal cycle of asexual spore maturation and abstriction in bean powdery mildew, Erysiphe polygoni, a non-chain former. The numbers presented represent the actual numbers of each binumerical conidiophore type observed at each period of collection. The type most frequently observed is indicated by bold-face numbers*

No. of cells in conidiophore exclusive of basal cell	Diurnal cycle of maturation and abstriction of asexual spores											
	← a.m. →						← p.m. →					
	2	4	6	8	10	12	2	4	6	8	10	12
1+5												
1+4												
1+3			1	5	5		1		1			
1+2	40	35	35	44	55	3	5	1	38	38	18	30
1+1	4			2	1		23	42	11	3	5	13
0+3						2	1					
0+2	4		4			32	43	1	1			
0+1		4	4				4		14	1		

TABLE 4.—*Diurnal cycle of maturation and abstriction of conidia in powdery mildew of *Euonymus japonicus*, a non-chain former. Data are presented as in table 3*

No. of cells in conidiophore exclusive of basal cell	Diurnal cycle of maturation and abstriction of conidia											
	← a.m. →						← p.m. →					
	2	4	6	8	10	12	2	4	6	8	10	12
1+5				1	1							
1+4	1		1	4					1			
1+3			1	1					2			
1+2	42	54	71	60	100	42	11	10	18	39	45	41
1+1	3	22	1	3			1	27	36	29	14	5
0+3			2			1	1					
0+2	5	3	1		1	43	34	10				
0+1	1	4					1				4	

A graphical summary of the diurnal cycles of sporulation (Fig. 2) of the powdery mildews observed reveals that the chain-forming powdery mildews *Erysiphe cichoracearum*, *Sphaerotheca pannosa*, and *Podosphaera leucotricha* are similar in that their high phase of conidiophore development came about 8 a.m. and their low phase came about 2 p.m. The curve of conidiophore development of the non-chain-forming *E. polygoni* and *Oidium euonymi-japonici* (Fig. 2, Tables 3 and 4) differs in height and in contour from the curves of the conidial chain-forming mildews, while the latter differ among themselves mainly as to height of the high phase, as measured binumerically.

Diurnal Cycle of Spore Abstriction as Shown by Dislodgement of Conidia

In Fig. 3 are recorded graphically the results of spore-catching experiments with *Erysiphe cichoracearum*. Between the hours of 10 a.m. and 1 p.m. there was abundant abstriction of conidia, as is shown by the differences between the count at 10 a.m. and that at 1 p.m. and by the rise in the line between those two points. The diurnal cycle of maturation and abstriction of conidia, which is indicated by these spore counts for the powdery mildew of sunflower, is substantially similar to the diurnal cycle obtained by microscopic examination.

NUCLEAR DIVISION IN THE CONIDIOPHORE

Examination of the stained conidiophores of *E. cichoracearum* from sunflower revealed the frequent occurrence of two nuclei in the basal cell, and of two nuclei in the cell above it. The results of counts of the frequency of occurrence of conidiophores with two nuclei in the basal cell and of conidiophores with two nuclei in the cell above it (Table 5) indicate that nuclear division takes place in both cells but is the more active in the basal cell. These data suggest that both the basal cell and the cell above it may function as generative cells, as is indicated by Foëx's¹² drawings. As many

¹² See footnote 8.

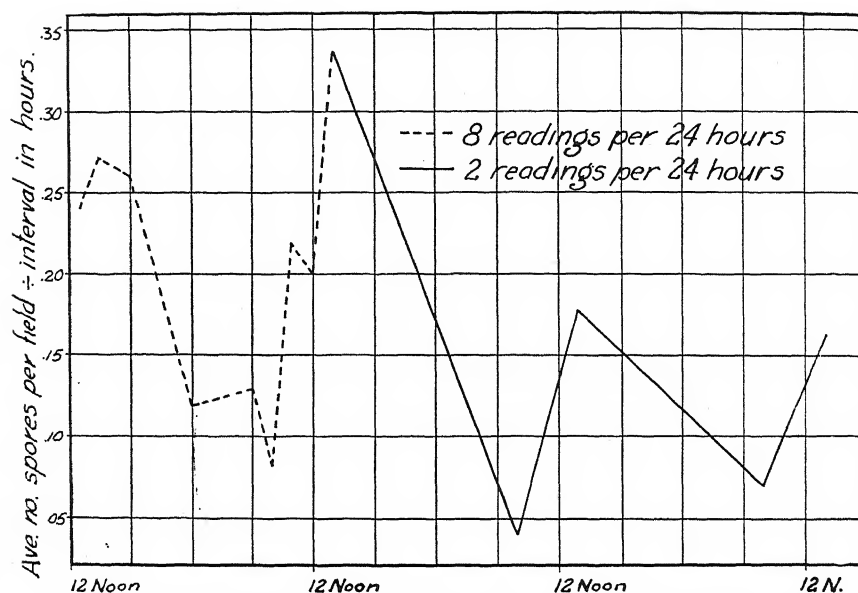


FIG. 3. Diurnal cycle of maturation of conidia of sunflower powdery mildew, *Erysiphe cichoracearum*. Each point on the graph indicates the number of conidia per low-power field per hour caught from the same excised leaves on 5 per cent sucrose. The leaves were snapped over a pint can at the bottom of which a glass slide had been placed. Each figure is based on from 125 to 175 microscope fields of 2.14 mm². From left to right an upward slope indicates abstriction of conidia and a downward slope indicates cessation or absence of maturation and abstriction.

as 4 nuclei in a single cell of a conidiophore have been observed, but this is considered unusual.

No pronounced diurnal cycle is apparent from the data of table 5, but it is noteworthy that, at the time of the experiment (December 3rd), the number of divided nuclei and recently divided cells observed for the daylight period (9 a.m. to 6 p.m.) was more than double the number observed for the night period.

TABLE 5.—Nuclear and cell division of *E. cichoracearum* on greenhouse sunflowers, Dec. 3, 1938. The tabulated figures represent the number of conidiophores, out of approximately 200 observed at each period, bearing the type of cell designated

Time of collection of conidiophores	Conidiophores with 2 nuclei in		Conidiophores showing recent divisions in	
	Basal cell	Second cell	Basal cell	Second cell
9 a.m.	7	8	2	8
12 midnight	12	5	1	10
3 a.m.	13	3	3	6
6 a.m.	6	3	0	7
Total (for dark period)	38	19	6	31
9 a.m.	30	19	11	18
12 midday	22	8	27	4
3 p.m.	12	7	9	5
6 p.m.	12	15	2	6
Total (for light period)	76	49	49	33

SUMMARY

Periodic microscopic examination of non-chain-forming powdery mildews of bean and *Euonymus* (*E. polygoni*-type conidiophores) revealed a diurnal cycle of conidiophore development similar to that reported for *E. polygoni* on clover. The period of abstriction of conidia occurred between 10 a.m. and 2 p.m. in both cases.

Periodic microscopic examination of the conidial-chain-bearing powdery mildews (*E. cichoracearum*-type conidiophore) of sunflower, rose, apple, aster, and cucumber revealed a more complex diurnal cycle of conidiophore development. Abstriction occurred between 6-8 a.m. and 2-4 p.m. and formation of the succeeding crop of conidia occurred between 2-4 p.m. and 6-8 a.m. in all cases.

In powdery mildew of the sunflower maximum spore abstriction occurred between 8 a.m. and 2 p.m. as shown by catching dislodged spores periodically over several days. A diurnal cycle of spore maturation and liberation, similar to that apparent from microscopic examination, was revealed.

Microscopic examination of stained conidiophores of sunflower powdery mildew revealed conidiophores with 2 nuclei in the basal cell and conidiophores with 2 nuclei in the cell next above the basal cell. This is believed to indicate that both cells may function generatively.

EXPERIMENTAL PRODUCTION OF BLACKFIRE ON TOBACCO¹

E. M. JOHNSON, STEPHEN DIACHUN, AND W. D. VALLEAU

(Accepted for publication Aug. 7, 1939)

It is common knowledge that natural or artificial inoculation of tobacco leaves with *Bacterium angulatum* produces only a small, relatively harmless, angular leaf spot, and inoculation with *Bacterium tabacum* usually produces only the so-called "typical" halo wildfire spot on well-nourished, rapidly growing tobacco plants. In field epidemics of late-season blackfire² caused by either of these organisms, the spots are large, zonate, and destructive, particularly on topped dark tobacco. For a long time it has been recognized that there is a close correlation between the occurrence of blackfire epidemics and rainy, stormy weather, but it also has been observed frequently that tobacco in low areas may be destroyed by blackfire whereas tobacco growing on somewhat higher land may escape injury nearly completely. Frequently this condition occurs without storm injury. Clayton^{3,4} has attempted to

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

² Blackfire is used in this paper to signify the concentric type of spot that occurs in wet seasons on topped dark tobacco. It may be caused by either *Bact. tabacum* or *Bact. angulatum*. The terms wildfire and angular leaf spot are reserved for the spots produced on tender tobacco leaves by the respective organisms. The etiological factors, aside from the parasite, which produce wildfire and angular leaf spot are so different from those causing blackfire that the latter may be considered a distinct disease.

³ Clayton, E. E. Water soaking of leaves in relation to development of the wildfire disease of tobacco. Jour. Agr. Res. [U. S.] 52: 239-269. 1936.

⁴ ———. Water soaking of leaves in relation to development of the blackfire disease of tobacco. Jour. Agr. Res. [U. S.] 55: 883-889. 1937.

show that water-soaking is a necessary factor for the development of what he has termed "epidemic" wildfire, and late-season blackfire. Valteau *et al.*⁵ have questioned this point, and have shown that water-soaking cannot explain the outbreaks of blackfire as they occur on maturing dark tobacco in Kentucky.

It is the purpose of this paper to report the experimental production of the typical, zonate, late-season type of spot in the field, and in the greenhouse under controlled conditions.

FIELD STUDIES

In 1935, large, zonate spots, typical of those occurring late in the season on both white Burley and dark fire-cured tobacco, were induced on white Burley tobacco in an infertile area on the Station farm at Lexington with single-colony and mixed cultures of *Bacterium angulatum*. The late-season disease was induced also on topped and suckered dark fire-cured tobacco in western Kentucky with cultures of *Bact. tabacum* and *Bact. angulatum*.

Prior to 1935 numerous field tests with *Bacterium angulatum* and *Bact. tabacum* were made on tobacco plants growing in fertile soil but produced only the typical angular leaf spots and halo wildfire spots. The 1935 inoculations with *Bact. angulatum* indicated that the fertility of the soil may have a marked effect upon the susceptibility of tobacco to injury by these organisms because inoculations to Burley plants in fertile areas of the same field developed the usual small, angular, relatively harmless spots.

In 1938, inoculations were made with *Bacterium tabacum* and *Bact. angulatum* to dark fire-cured tobacco throughout the season in an infertile area in western Kentucky. Late in the season, after topping, similar inoculations were made in plots of high and medium fertility at Lexington. In the western Kentucky tests 37 plants of the 60 inoculated with *Bact. tabacum* developed the large, zonate spots, typical of those occurring in nature on topped dark tobacco (Fig. 1). On the same plot 3 plants of 60 inoculated with *Bact. angulatum* developed the late-season zonate disease. At Lexington, in the plot of medium fertility, 8 of 9 plants inoculated with *Bact. tabacum* and 2 of 9 plants inoculated with *Bact. angulatum* developed $\frac{1}{4}$ to $3\frac{1}{2}$ inch zonate spots. In the fertile plot at Lexington 3 of 9 inoculated with *Bact. tabacum* and 2 of 10 inoculated with *Bact. angulatum* developed the destructive zonate spot.

During the tests in western Kentucky rains fell on June 10, 11, 18, 19; July 2, 12, 17, 18, 19, 29, 30, 31; August 2, 3, and 11. The rains in June were not heavy and were unaccompanied by wind. Those of July 2 and 12 were heavy and were accompanied by high north winds. Despite the heavy and frequent rains of July and August water-soaking of leaves was never observed even though the winds broke many leaves. Leaves on the windward side of plants were often turned over and had on their under surfaces $\frac{1}{8}$ to $\frac{1}{2}$ inch bruised areas made up of numerous pinpoint water-soaked appear-

⁵ Valteau, W. D., S. Diachun, and E. M. Johnson. Injury to tobacco leaves by water soaking. *Phytopath.* 19: 884-890. 1939.

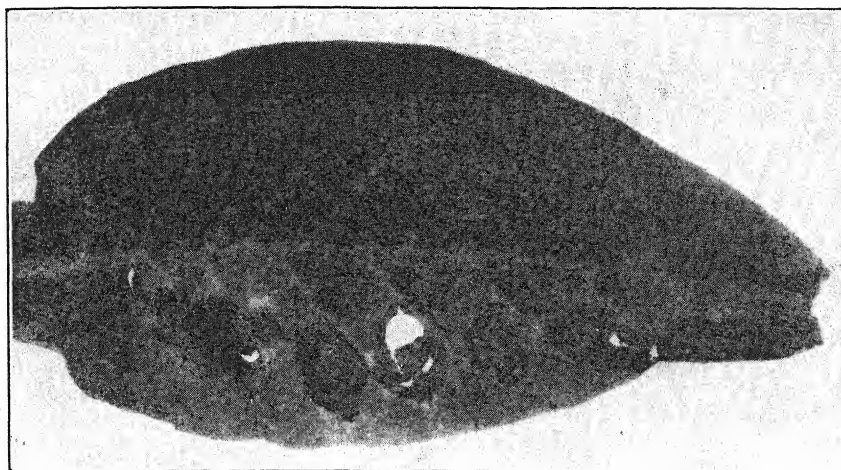


FIG. 1. Fourth leaf from the bottom of a topped plant of dark fire-cured tobacco, atomized in the field with *Bacterium tabacum*, June 15, 1938. (Dew was heavy every morning. Rain fell, June 16 and 18.) Photographed June 30, 1938.

ing spots (stippling), which usually disappeared in 3 to 8 hours. This stippling is not to be confused with incipient infections sometimes found around older spots. Many of these leaves were tagged and some were inoculated with *Bacterium tabacum* and *Bact. angulatum*. Comparable leaves without stippling were tagged and some were inoculated. The stippled leaves developed no more natural infection, nor did they develop more infection when inoculated than the nonstippled leaves.

During most of the tests, both in western Kentucky and at Lexington, dews were heavy and the plants remained wet until 9 or 10 a. m. On inoculated plants the dead centers of spots were, in early morning, surrounded by a narrow border of dark green to brown, wet, necrotic tissue sharply delimited from the inner dead, brown tissue and the outer, healthy area. During the day this wet, necrotic border dried out; if there was bright sunlight, it had a scalded appearance. In this way, spots gradually increased in size from day to day and coalesced, the final size often being limited only by contact with the midrib or larger veins. Heavy dews seem to be important not only in the increase in the size of spots but also in the development of new infections. On plants inoculated in the field, in the absence of rain, new infections have been observed on leaves directly under inoculated areas, during periods of heavy dews. It has been observed repeatedly that low areas in fields, in seasons of little rainfall, may develop blackfire, whereas the high areas may be entirely free. Plants in these low areas have been observed to remain wet much longer in the morning than those on the higher areas. Fogs may be important. It is not unusual to observe fog pockets in the early evening in many fields.

GREENHOUSE STUDIES

In conjunction with the field studies, experiments were carried on in the greenhouse with low-topped Burley and dark tobacco plants inoculated with

pure cultures of virulent single-colony isolates of *Bacterium tabacum* and *Bact. angulatum*. Two preliminary experiments showed that wildfire infection on untopped and topped plants kept dry was of the typical halo kind, while spots on topped plants kept in an artificial fog produced by an atomizing spray nozzle operated by steam were larger, were surrounded by a dark green-gray, wet border, and usually were without halos. These spots increased in size and often coalesced to form large, dead areas made up of several concentric spots. If the inoculated topped plants were kept in the fog continually the spots were not zonate, but if kept in the fog during the night and allowed to dry in the daytime zonate bands developed.

On November 29, 1938, three low-topped Burley plants and three untopped plants of the same age, in 8-inch pots, were inoculated with 24-hour broth cultures by atomizing; the atomizer was held $\frac{3}{4}$ of an inch from the lower leaf surface. The left side of each leaf was inoculated with *Bacterium angulatum* and the right side with *Bact. tabacum*. Two topped and two nontopped plants were placed nightly from 5 p. m. until 8 a. m. in the fog, which kept the leaf surfaces moist. Water-soaking was never observed on these plants. A topped and a nontopped plant were placed at one end of the greenhouse away from the fog. By December 5 infections caused by both *Bact. tabacum* and *Bact. angulatum* were destructive on the 3 upper leaves of the 2 plants kept in the fog nightly. Spots were coalescing to form dead areas $\frac{1}{2}$ to 1 inch in diameter. There was but little difference between the spots caused by *Bact. tabacum* and those caused by *Bact. angulatum*. On the nontopped plants in the fog and the plants kept dry both wildfire and angular leaf spots were of the typical relatively harmless type.

By December 6 some of the spots on the topped plants kept in fog nightly showed two or three distinct zones, the outermost one being a necrotic, wet border.

Every night until December 10, when the experiment ended, another band or zone was added to the spots on the topped plants kept in the fog at night. In the morning the new zone was wet, necrotic, grey-green or light brown, and not sunken. During the day, when the spray was turned off and the leaf surface became dry, the zone became dry, sunken, and brown, usually with a sharp dark brown line of demarkation between the dead tissue and the healthy green tissue, with but little if any increase in size of the spots during the day.

The necrotic spots on the topped plant not in the fog were not so large as those on the topped plants kept in the fog; they were not zonate. The spots on all the nontopped plants were of the familiar typical halo wildfire, and small angular-leaf-spot type.

This experiment was repeated on January 4, with similar results. In figure 2 is shown the actual daily increase in size of spots produced when leaves inoculated with *Bacterium angulatum* and *Bact. tabacum* were placed in the fog during the night and allowed to dry off during the day.

Twice, low-topped dark-fired plants were atomized with *Bacterium tabacum*. Both times, large, zonate spots, such as those shown in figure 3 de-

veloped on the plants kept in the fog. In one of these experiments one side of each leaf was atomized with *Bact. angulatum*. The resulting infections developed much more slowly than the spots on the same leaves caused by *Bact. tabacum*, and only a few of the spots on the side of the leaves inoculated with *Bact. angulatum* became large and zonate.

Two experiments were performed in which inoculation with *Bacterium tabacum* and *Bact. angulatum* was made by needle prick rather than atomizing. In neither case were such inoculations very successful in producing blackfire but a small per cent of the infections did produce rather large, zonate spots.

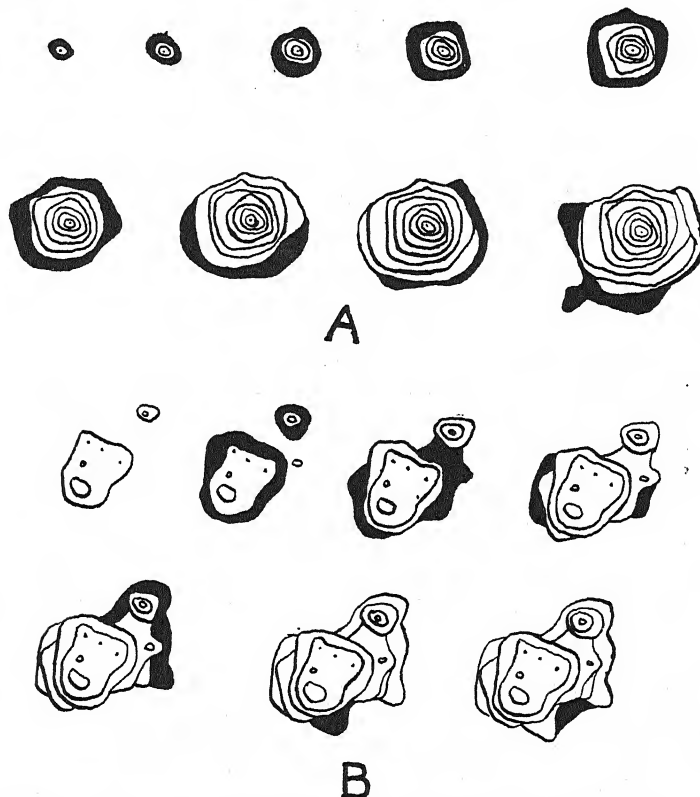


FIG. 2. A. Daily increase in a typical zonate blackfire spot produced by *Bact. angulatum* when the inoculated plant was placed in a fog nightly. The black outer zone was necrotic and wet in the morning, when the drawing was made. B. Daily increase in size of a zonate blackfire spot produced by *Bact. tabacum* under the same conditions.

DISCUSSION

Although it has been believed for some time that rainy weather is in some way connected with the rapid development of blackfire outbreaks in the field, the zonate blackfire type spot has not previously been produced experimentally by inoculation with pure cultures of either *Bacterium angulatum* or *Bact. tabacum*. Consequently it was not definitely known that either of these organisms was necessarily concerned in outbreaks of the disease on topped dark tobacco, and the relation of wet weather to outbreaks was not

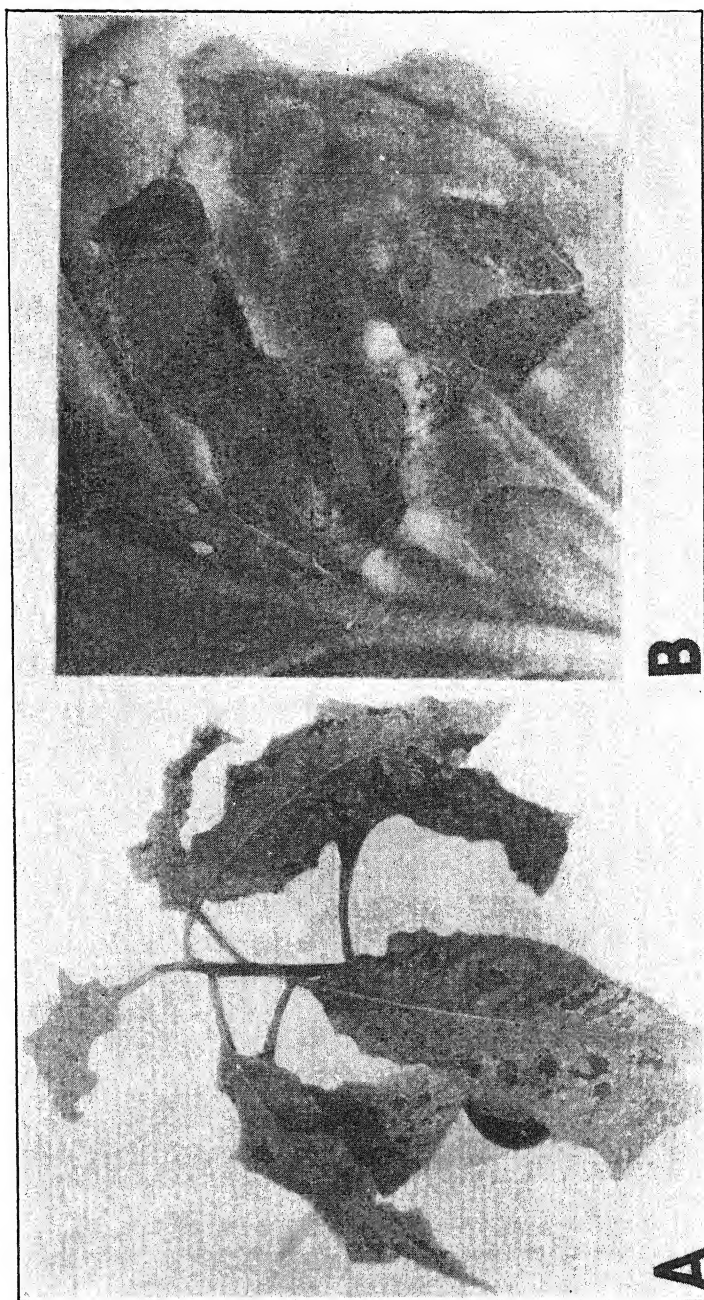


FIG. 3. A. Blackfire on a topped dark tobacco plant atomized with *Bact. tabacum* 6 weeks after topping. After inoculation the plant was kept in a fog nightly. Photographed 11 days after inoculation. B. Close-up view of several zonate spots on the same plant showing coalescence of spots. Photographed 22 days after inoculation.

clearly understood. The theory has been advanced⁶ that water soaking of leaf tissues during storms is necessary to break down the resistance of tobacco leaves to *Bact. tabacum* and *Bact. angulatum* in the natural development of the disease in the field. But our observations during outbreaks of blackfire on dark tobacco have indicated that, whereas there might sometimes be a marked increase in injury following a severe storm, yet the zonate blackfire spots frequently developed in the absence of storm injury and in the absence of conditions likely to bring about water soaking of the leaves. Furthermore, isolation studies from the advancing edges of blackfire spots showed these tissues to be largely sterile. The experiments here reported show that large zonate spots can be produced on low-topped Burley and dark tobacco plants in the field, and in the greenhouse, in the absence of water-soaking. In these experiments it was found that if leaves atomized with a virulent isolate of *Bact. tabacum* were kept moist during a part of the day by placing the plants in an artificial fog, large, zonate spots typical of those occurring in natural epidemics were produced.

Thus far, only relatively virulent isolates of the organisms have been used. Whether weak strains would produce the same results is not yet known. In these studies *Bacterium tabacum* usually caused larger and more destructive spots than *Bact. angulatum*. Sometimes *Bact. tabacum* produced large spots when *Bact. angulatum* failed to do so on another part of the same leaf. It may be that the isolate of *Bact. angulatum* used was not sufficiently virulent, and that more virulent strains might be more nearly comparable to the virulent isolate of *Bact. tabacum* in the production of large zonate spots.

SUMMARY

Large, zonate spots very similar to those occurring in natural late-season epidemics of blackfire were produced on Burley and dark tobacco plants in the field by atomizing leaves with *Bacterium tabacum* and *Bact. angulatum*. Dew usually covered the inoculated leaves every morning.

Similar large, zonate, destructive spots were produced on topped Burley and dark tobacco plants inoculated with virulent isolates of *Bacterium tabacum* and *Bact. angulatum* when the plants were kept nightly in an artificial fog which formed a film of water on the leaves.

Both in the field and in the greenhouse the spots increased in size during the night, when a wet, necrotic, advancing border was formed that became dry and brown during the day.

Large dead areas 3 to 4 inches in diameter were formed by increase in size of individual spots and coalescence of adjacent spots.

This appears to be the first report of the experimental production of the zonate blackfire spots by pure culture inoculations and under controlled conditions.

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⁶ E. E. Clayton. *Loc. cit.*

TIME OF GROWTH¹ OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA AT RHODODENDRON, OREGON

J. W. KIMMEY

(Accepted for publication July 14, 1939)

INTRODUCTION

The seasonal fluctuations in growth rate of white pine blister rust (*Cronartium ribicola* Fischer) cankers on western white pine (*Pinus monticola* Douglas) were studied at Rhododendron, Oregon, from 1934 to 1937, inclusive. Lachmund² and Buchanan³ determined the annual growth rate of blister rust cankers in British Columbia and Idaho, and Lachmund² found that canker growth is less in winter than during the growing season. Rhoads⁴ observed that growth of the cankers on *Pinus strobus* L. during the latter part of the summer was twice as fast as that during the spring and early part of the summer of 1918 at Kittery Point, Maine. The study herein reported was made to secure information on the time of year and at what relative rates growth of blister rust cankers takes place, and thereby to gain a better conception of the phenology of this important parasite.

THE STUDY AREA

The area selected for the study is on the west slope of the Cascade Mountains, about 10 miles southwest of Mount Hood, at Rhododendron, Oregon. This location is approximately at the latitudinal center of the botanical range of *Pinus monticola*, and at this latitude the species ranges from near sea level to about 5,000 feet elevation. The study was made in a typical stand of reproduction from 10 to 20 feet in height, growing on nearly level ground at an elevation of approximately 1,650 feet and well within the natural range of western white pine in that region. The trees used were typical in thrift for the locality and of average size for the stand.

METHODS

Cankers were chosen for study by selecting as nearly as possible a relative representation of all cankers occurring on the trees used. That is, an equal percentage of existing branch cankers and stem cankers were chosen, and also a proportional number of existing primary- and secondary-branch cankers. The numerical basis employed in each diameter class was approximately proportional to the natural frequency of cankers as found on the

¹ The term "growth" of cankers is used to designate the extension of the discoloration of the bark, which accompanies and bears a fairly constant relation to the growth of the mycelia in the bark.

² Lachmund, H. G. Growth and injurious effects of *Cronartium ribicola* cankers on *Pinus monticola*. Jour. Agr. Res. [U.S.] 48: 475-503. 1934.

³ Buchanan, T. S. Annual growth rate of *Cronartium ribicola* cankers on branches of *Pinus monticola* in northern Idaho. Phytopath. 28: 634-641. 1938.

⁴ Rhoads, Arthur S. Studies on the rate of growth and behavior of the blister rust on white pine in 1918. Phytopath. 10: 513-527. 1920.

area. An effort was made to secure cankers that were of average health and vigor, that would remain alive for at least one year, and that would not coalesce with another canker before the experiment was completed. Cankers in all stages of development, from the first incipient discoloration to those that had produced aecia several times, were used. Cankers were selected in all parts of the crowns in an effort to obtain a true sample of those subjected to all existing local influences.

At the start of the experiment the limits of discoloration on each canker were marked with paint, as described by Buchanan,⁵ and the following data were recorded for each canker: Canker number; type of branch or stem; location in the tree; the year's growth upon which the canker originated; the total length of the canker; the diameter of the branch or stem at both extremities of the canker; the stage of development; and the general condition of the canker. These data were used both in judging the general representation of the samples and in attempting correlations of probable influences with differences in time of growth for individual cankers.

Growth measurements were secured on two series of cankers. In Series 1, 52 cankers, on 12 trees, were marked on March 8, 1934, and were measured periodically until June 5, 1935. In Series 2, 65 cankers, on 7 trees, were marked on March 6, 1936, and measured periodically until May 12, 1937.

In preparation of the study plan it was believed that growth measurements taken at monthly intervals⁶ throughout the year would be sufficient to show the time of growth. Soon after the experiment was under way, however, it became evident that at certain times in the year growth measurements at shorter intervals would be necessary. Accordingly, measurements were taken at 2-week intervals on the cankers of Series 1 from early November, 1934, to early June, 1935. The cankers in Series 2 were measured at weekly intervals from the time of marking to early May; and at monthly intervals thereafter, except during the periods of growth retardation in the fall of 1936 and of the beginning of growth acceleration in the spring of 1937, when measurements were taken at weekly intervals.

Just as in similar studies by Lachmund and Buchanan⁷ the extremities of the cankers were considered to be at the limits of the discoloration of the infected bark. At the time of each measurement the total growth, since the time of marking, toward the distal end of the branch or stem was considered as growth upward, and the total growth, since the time of marking, toward the proximal end as growth downward. Growth measurements were carefully taken with vernier calipers registering to the nearest hundredth of an inch.

RESULTS

There were great differences in time of growth between individual cankers. All cankers ceased perceptible growth for a period of at least 1

⁵ See reference in footnote 3.

⁶ The intervals between measurements were not always exactly one month, two weeks, or one week, but were approximately so.

⁷ See references in footnotes 2 and 3.

month during the year. Some stopped growing as early as September, and others continued growth until December. Most cankers showed no growth during January. However, there was no period of a month's duration during which all cankers showed no growth. Appreciable growth usually commenced in late March, but many cankers showed no growth until April and some did not grow until May. The individual canker that grew continu-

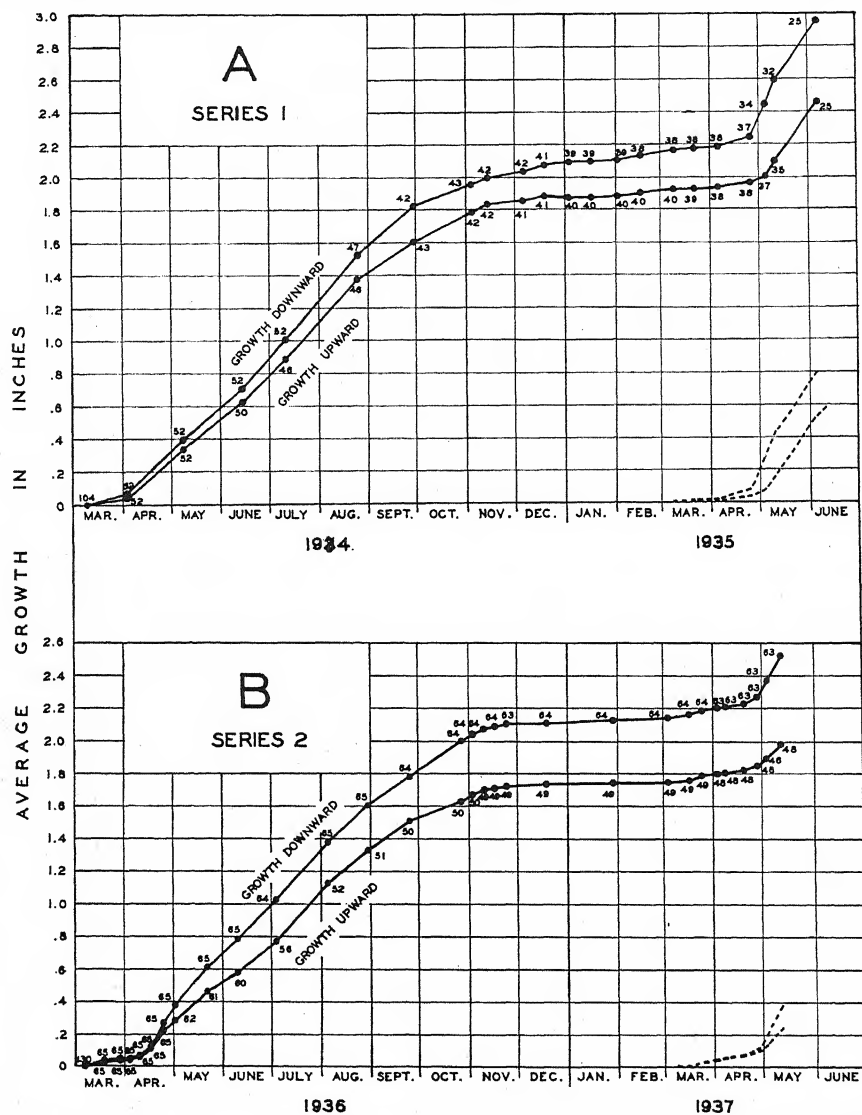


FIG. 1. The average cumulative growth, both upward and downward: A, for cankers in Series 1 during the course of observations from March 8, 1934, to June 5, 1935; and B, for cankers in Series 2 from March 6, 1936, to May 12, 1937. The dates on which measurements were taken are indicated by the positions of the points on the graph, and the small numeral at each point gives the number of cankers on which it was based. For convenience in comparing spring growth, the terminal portions of the graphs are repeated, by dotted lines, starting at zero on March 8, 1935, and March 8, 1937.

ously for the greatest number of months showed detectable growth for a period of 11 months, while at the other extreme 1 canker showed no growth for a period of 6 months. To a lesser extent differences were found between the time of growth of the 2 ends of individual cankers. For example, cankers were noted on which upward growth stopped in the early fall, while growth downward continued slowly for a month or more.

The data were analyzed in an effort to correlate peculiarities of time of growth of individual cankers with the various factors that were believed to have some influence on time of growth. No apparent correlation was found between time of growth of individual cankers and any one of the following factors: Age of canker; stage of canker development; general health of canker; type of canker (stem cankers, primary-branch cankers or secondary-branch cankers); size of cankered branch or stem; location of canker in the tree; or size of tree.

The seasonal fluctuations of canker growth are illustrated in figure 1, which shows graphically the growth, both upward and downward, for cankers in both series during the course of the observations.

In figure 1 it may be noted that after the start of a series certain cankers were eliminated from the basis. The principal cause of cankers' becoming

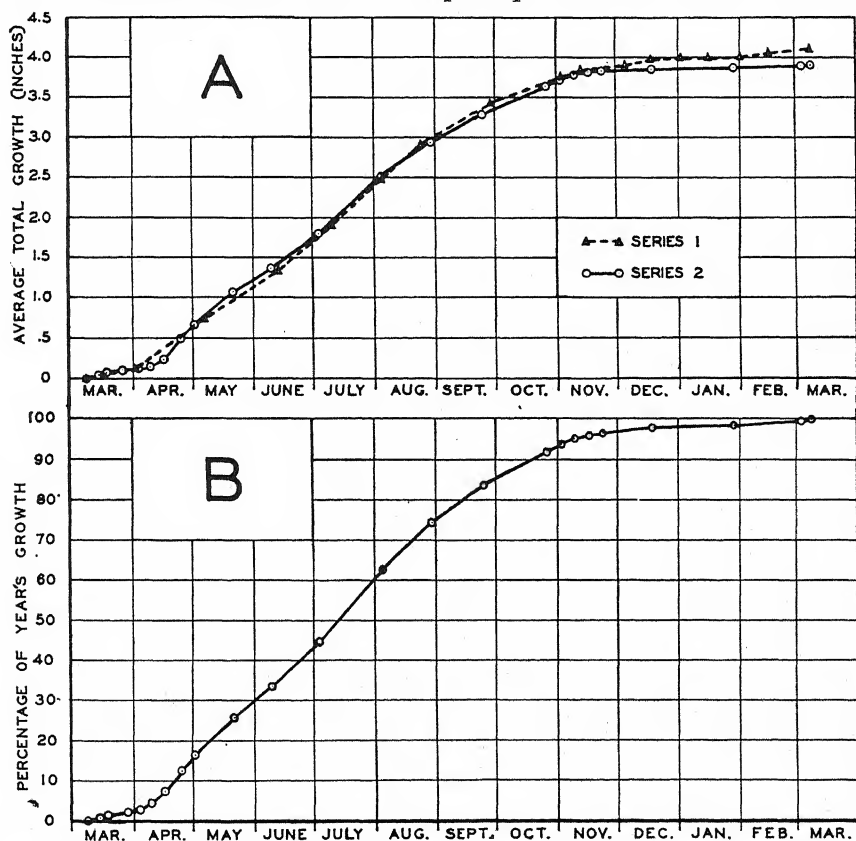


FIG. 2. Growth of cankers from March 8 to March 8: A, average cumulative total growth in each series; B, average cumulative percentage of total growth in both series.

unusable, especially for measurements of upward growth, was "flagging" (death of the branch beyond the canker). Two other important causes were coalescence with other cankers and growth of cankers into nodes or whorls where the discoloration limits could not be seen. Certain small irregularities in the graphs may be accounted for by these changes in the bases.

The combination of upward growth and downward growth is described as the total growth of a canker. The average cumulative total growth of cankers for Series 1 is compared with that of Series 2 in figure 2, A, showing the similarity of growth fluctuations and amount of yearly growth for the two series. In figure 2, B, the two series are combined to show the average cumulative percentage of the total growth for a 1-year period.

DISCUSSION

The period over which canker growth occurred is shown in figures 1 and 2. All of the graphs show that the growth was greatest from April to November and that there was little growth during the winter months. From figure 2, B, it may be seen that 90 per cent of the year's growth took place from April 1 to November 1, and that only about 2.5 per cent of the year's growth occurred in December, January, and February. Most of the cankers showed no growth during the winter months. Although the average curve shows a slight growth throughout the winter, each of the cankers stopped perceptible growth for at least a month during the winter and most of them for 2 or 3 months or longer. The results agree with Lachmund's⁸ findings that canker growth on *Pinus monticola* is less in winter than during the growing season. Rhoads'⁹ observations that canker growth on *P. strobus* during the latter part of the summer was twice as fast as that during the spring and early part of the summer are in contrast with the findings for *P. monticola* reported by Lachmund, as well as the results herein reported.

One factor that showed some apparent correlation with the time of canker growth was temperature. Although no daily temperature records are available for the immediate vicinity of the study area, the general monthly records for the region indicate that temperature was one of the factors governing the period over which canker growth occurred. The weather records show that the spring of 1934 was unusually warm. Following the mildest December of record, January, February, March, and April, all established new records for high mean temperature. The effect of this unusually warm spring is manifested in the growth curves of figure 1 when comparison is made with the more nearly normal weather of the 3 following springs. The springs of 1935 and 1937 were somewhat below normal in temperature. January of both these years was unusually cold—January, 1937, being the coldest month of record in Oregon. These cold periods in January caused a severe check of vegetative growth in both years. In 1935 the beginning of spring was rather cold. March was only 1° warmer than February, and both March and April had temperature below normal, that of April being the lowest mean for that month since 1929. April of 1937

⁸ See reference in footnote 2.

⁹ See reference in footnote 4.

was cool and unusually cloudy, having the greatest number of cloudy days of any April since 1915. The effect of this cool weather in April of both 1935 and 1937 may be seen on the graphs in figure 1 when compared with the nearly normal spring of 1936 and the unusually warm spring of 1934.

The falls of 1934 and 1936 were both somewhat warmer than normal, although a sudden cold period, commencing the first of November in 1936 and lasting most of that month, shows some effect on the period of rapid canker growth as depicted by the graphs in figure 1. (Compare the sudden leveling off in November of the growth curve for 1936 with the gradual leveling off of the growth curve for the fall of 1934.)

In each of the 4 springs during the course of this study, the breaking of peridia on the aecia-producing cankers throughout the general vicinity synchronized very well with the beginning of appreciable growth of cankers of all stages. In each case perceptible growth started at about the time the first peridia were broken, and by the time most peridia were broken rapid canker growth was under way. Another consistent indicator of time of canker-growth acceleration was the breaking of buds on deciduous trees in the vicinity of the study area. Rapid canker growth ceased in the falls of both 1934 and 1936 at about the time the deciduous trees in the vicinity dropped their leaves. It is believed that these indicators of the beginning and the end of rapid canker growth may be applicable in estimating the period of rapid canker growth in other localities.

SUMMARY

A total of 117 white pine blister-rust cankers on 19 western white-pine trees were used in a study of the seasonal fluctuations of canker growth rate at Rhododendron, Oregon. Two series of cankers were used, one series in 1934 and 1935 and the other in 1936 and 1937. Canker growth curves are shown. Great differences in time of growth were found between cankers, and to a lesser extent between the two ends of individual cankers. Growth was rapid from April to November and ceased for 1 to 3 months or more during the winter. About 90 per cent of the annual growth took place from April 1 to November 1, and only 2.5 per cent occurred during December, January, and February. Temperature is believed to have been one of the factors governing the time of rapid canker growth. The time of breaking of the peridia on blister rust cankers and the time of the breaking of buds of deciduous trees in the spring are considered as probable indicators of the time of canker growth acceleration, while the time of leaf-cast of deciduous trees in the fall is suggested as a probable indicator of the time of canker-growth retardation.

BRANCH OFFICE OF THE

DIVISION OF FOREST PATHOLOGY,

BUREAU OF PLANT INDUSTRY,

MAINTAINED AT PORTLAND, OREGON, IN

COOPERATION WITH FOREST SERVICE,

U. S. DEPARTMENT OF AGRICULTURE.

PHYTOPATHOLOGICAL NOTES

*A Method for Testing Resistance of Tomatoes to Fusarium Wilt.*¹—Many tests have been made of the varietal susceptibility of tomatoes to *Fusarium bulbigenum* var. *lycopersici* Wr. and R. for the purpose of selecting wilt-resistant varieties and strains adapted to particular conditions. These tests usually have been conducted under field conditions in soil known to be infested with the wilt organism. Edgerton² and, later, others planted directly in naturally or artificially infested soil, to eliminate susceptible individuals. In many cases the seedlings have been transplanted to infested soil in trays or cold frames. This technique permits the study of a larger number of plants than would be possible if they had been grown in clean soil and set in infested fields. Wager³ and others have inoculated tomato plants by placing mycelium in holes at the base of the plants. This, however, is laborious and requires large amounts of inoculum if many seedlings are to be inoculated.

A method of inoculating thousands of young tomato plants was used with marked success in the spring of 1939 at the Tomato Disease Laboratory at Yoakum, Texas. The roots were immersed for periods of from 5 to 10 minutes in 4-7-day-old liquid nutrient cultures of *Fusarium bulbigenum* var. *lycopersici*. The cultures had been agitated daily to insure uniform distribution of the fungus through the inoculum. The plants were immediately transplanted to flats or cold frames. All tests were conducted out of doors from February through May. In the first experiment, seven-week-old plants from a large number of resistant and susceptible varieties were inoculated in this manner on February 24. The first definite symptoms were observed on March 15. Of the 1200 plants used in this test only 33 lived long enough to produce any seed and none of them were free of the vascular discoloration so characteristic of tomato wilt. Many of the resistant commercial varieties were included in this test. The reaction of the susceptible Gulf State Market and the resistant Louisiana Pink and Louisiana Dixie varieties is illustrated in figure 1. All of the plants in Gulf State Market variety have succumbed, while most of the plants in the other two still are apparently healthy. However, many of these plants later died from wilt.

Two other tests were conducted in which the roots of 8255 young plants also were immersed in a culture of the wilt fungus. There were 168 different samples of tomatoes, most of them being standard varieties. A few, however, were from a collection made by H. L. Blood, in South America, in 1937-38, and obtained through L. R. Hawthorn, horticulturist at the Winter Haven substation. The first distinct symptoms of tomato wilt appeared in 10 days in one test and in 16 days in the other. The relative resistance of the different varieties and strains was obtained by making counts at 10-14-

¹Published with the approval of the Director as contribution No. 544, Technical Series, of the Texas Agricultural Experiment Station.

²Edgerton, C. W. A study of wilt resistance in the seed bed. *Phytopath.* 8: 5-14. 1918.

³Wager, V. A. *Fusarium* wilt in tomatoes in South Africa. *So. Afr. Jour. Sci.* 30: 240-246. 1933.

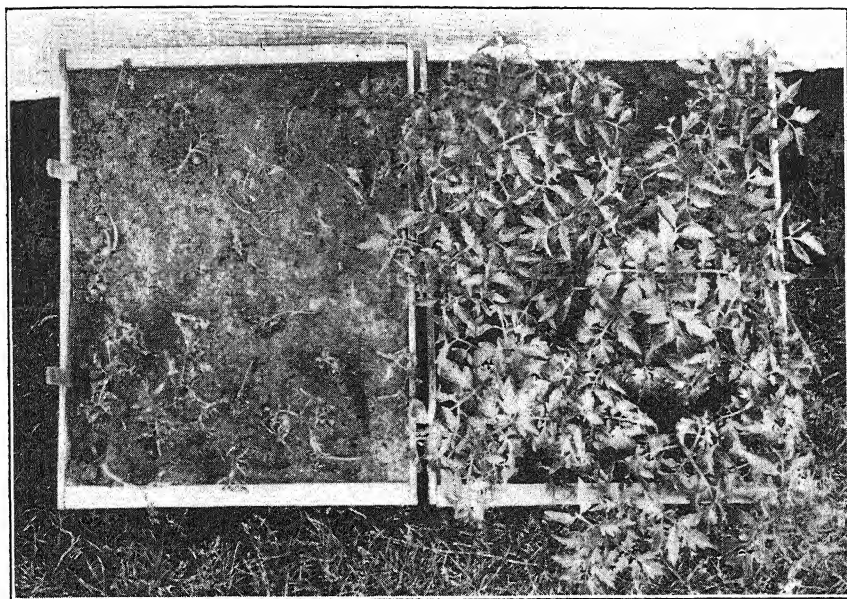


FIG. 1. Photograph taken 25 days after inoculating young tomato plants by immersing the roots for 10 minutes in a liquid nutrient culture of *Fusarium lycopersici*: Two strains of Gulf State Market in the tray on the left, Louisiana Dixie and Louisiana Pink in the tray on the right. Only 6 plants from this tray produced seed and they were of the Louisiana Pink variety.

day intervals. The plants were classified according to the severity of the disease. On the final count the stems of those plants not showing pronounced symptoms were cut and examined for internal discoloration. Only 5 plants from the standard varieties failed to show the vascular discoloration, while a number of plants in several of the South American lots were free of the disease.

The wilt-resistance ratings obtained from these counts were compared with the ratings obtained under severe wilt conditions in the field. In nearly all cases the ratings were in the same order, except that those for the plants artificially inoculated were generally somewhat lower, indicating that this method may be a more selective one than the setting of plants in infested fields.

The technique herein described for inoculating tomato plants is proposed as an aid in the selection of wilt-resistant tomatoes and in the study of strains of the fungus, since it is simple, accurate, and rapid. Readings on wilt resistance can be made within 4 weeks after inoculation if conditions are favorable for the development of tomato wilt. The method is similar to the one mentioned by Weindling and Armstrong⁴ for inoculating cotton with the cotton wilt fungus.—A. L. HARRISON, Tomato Disease Laboratory, Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station, A. and M. College of Texas, Yoakum, Texas.

⁴ Weindling, R., and Armstrong, G. M. A water-culture infection method used in the study of *Fusarium* wilt of cotton. *Phytopath.* 29: 23. 1939.

Unusual Bacterial Spot Symptoms on Peach Leaves.—An aggravated case of the bacterial spot disease (*Bacterium pruni* E. F. S.), with unusual leaf symptoms, was observed near Springdale, Arkansas, on June 1, 1939. All stages of leaf-spot development were present in the 18-month-old Elberta orchard, but, instead of culminating in the typical shot-hole effect, large areas of the leaves became completely infiltrated with bacteria (Fig. 1, A). These affected areas, in some cases involving as much as one-half of the leaf, had a greenish-yellow translucent appearance when viewed by transmitted light, but were dark brown under reflected light. The ground beneath the trees was littered with leaves showing these atypical bacterial-spot symptoms, and other affected leaves (Fig. 1, B) remained in the trees, detached from the twigs, but adhering to adjacent leaves by the mixture of gum and bacteria, which oozed from the affected portions.

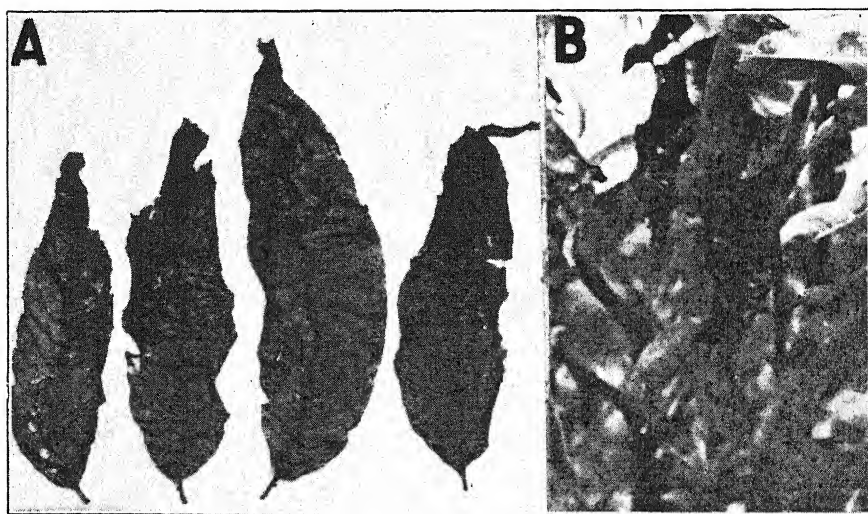


FIG. 1. A. Elberta peach leaves with different amounts of the leaf area invaded by *Bacterium pruni*. B. Diseased leaves attached to other leaves by the mixture of gum and bacterial exudate, which oozed from the infiltrated tissues.

Microscopic examination of the infiltrated, translucent areas revealed a complete disorganization of the cellular structure of the leaf. The small veins, which ordinarily delimit the size and shape of the spots, did not act as barriers to the spread of the organism. Pure cultures of the organism were secured from the infiltrated tissues.

These unusual symptoms were much more pronounced in two-thirds of the 20-acre block, where string beans had been grown between the peach rows in 1938 and the dried bean vines had been plowed under in March, 1939. In the balance of the block, where the beans had not been planted in 1938, the disease, while present, was running a normal course with only moderate defoliation and only an occasional leaf showing the infiltrated areas.—JOHN C. DUNEGAN, Fayetteville, Arkansas, Division of Fruit and Vegetable

Crops and Diseases, U.S.D.A., Washington, D. C., cooperating with the Arkansas Agricultural Experiment Station.

A Blight of Wild Cherry Seedlings.—A blighting of wild cherry (*Prunus serotina* Ehrh.) seedlings has been under observation since 1924. The disease, caused by *Sclerotinia seaveri* Rehm, appears each spring about the time the second pair of true leaves unfolds. The first symptom is the development of a brown, water-soaked region near the apex of the stem. This condition is accompanied by a loss of turgor and the infected seedlings (Fig. 1) are

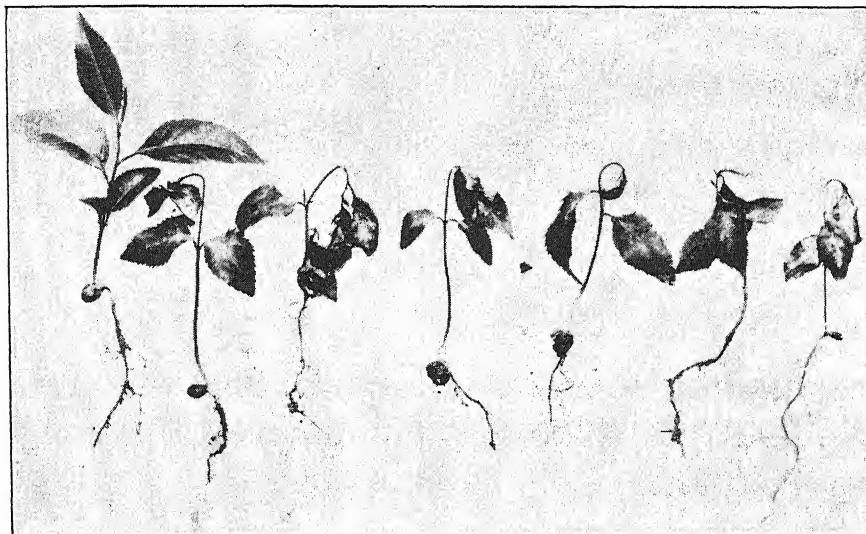


FIG. 1. Blighted wild-cherry seedlings showing the characteristic drooping of apical portion of stem. Healthy seedling at the left.

readily detected by the characteristic drooping of the affected portion of the stem. The infection spreads from the stem into the leaves through the petiole and mid-rib. The basal portion of the leaf turns brown and finally the whole leaf is affected, assuming a bleached grey color. Conidial masses frequently develop on the leaves. The fungus continues to spread down the stem and, when it reaches the ground line, the young plant dies.

The disease was observed at Fort Valley, Georgia, from 1924 to 1928 and subsequently has been observed in the vicinity of Fayetteville, Arkansas.

TABLE 1.—Number of wild-cherry seedlings blighted in three quadrants on April 4, 1928

Quadrant number	Total number seedlings	Number healthy	Number blighted	Per cent blighted
1	147	55	92	62.5
2	88	58	30	34.0
3	250	149	101	40.0

In 1928, 3 quadrants, each 1 sq. m. in area, were laid out at random under a large tree near Fort Valley, Georgia. Although the total number of seedlings blighted during the period of seedling germination was not ascertained, the counts made on April 4 (Table 1) show from 34 to 62.5 per cent of the seedlings affected on that date. It is evident from these figures that the disease must be considered as a factor limiting the reproduction of *Prunus serotina* in the South.—JOHN C. DUNEGAN, Fayetteville, Ark., Division of Fruit and Vegetable Crops and Diseases, U.S.D.A., Washington, D. C., cooperating with the Arkansas Agricultural Experiment Station.

BOOK REVIEWS

MELHUS, IRVING E., AND GEORGE C. KENT. *Elements of Plant Pathology*. 493 pages (including glossary, list of books and index), 259 figures. The Macmillan Company, New York. 1939. \$4.00.

This book, printed in larger type and on whiter paper than most texts of recent date in the field of applied botany, is timely in its appearance and of more than passing interest for the plant scientist and student. As stated by the authors in their Preface, "The chief contribution of the book lies in the emphasis placed on parasitism in disease processes and the principles relating to control measures, coupled with the condensation and omission of unnecessary morphological and mycological data." The text was not prepared to answer, nationwide, the needs of instructors in plant pathology but, on the other hand, it should be on the reference shelf of all research workers and teachers in botany and plant pathology and available to all students in these fields of science. As a text book for use in colleges and universities, it could be appropriately adopted over a large part of the United States with very little supplementation or adaptation.

It is approximately $6 \times 8\frac{1}{2} \times 1\frac{1}{2}$ inches in size, substantially bound in plain green buckram, stamped on back and cover with the title in gold letters on a dark rectangular background. The contents consist of an introduction and 15 additional chapters dealing with plant pathology, 6 pages of glossary, a list, covering more than 4 pages, of books including author, title, publisher, year, and number of pages, dealing with some phase of plant pathology, and 17 pages of index in which bold-face type indicates illustrations. About 20 per cent of the text figures (51 of a total of 259) are diagrams of the host relation of the various parasites, indicating the active and dormant parts of the life cycles of the parasites and, in most instances, their parasitic and saprophytic rôle. These figures are of particular interest to the student in illustrating relationships where more than one spore form exists and where alternate hosts are included. The line drawings are well chosen and excellently reproduced, whereas the remaining illustrations, generally good, too frequently indicate lack of good material from which to choose and poor reproduction, resulting in difficulty of interpretation by the student.

The first 21 pages deal with introductory remarks and more or less of the historical background of this science, which is further developed in the next 46 pages under the headings of symptoms, parasitism and influences of environment on plant disease. The principles of control measures are set forth in an equal number of pages where rotation, sanitation, protection, quarantine, eradication, and resistance are described in varying degrees of detail. The first, second and third are considered under temporary and the remaining three methods under permanent control. The information on disease control is up-to-date with historical jottings and tabular data. The chemical reactions may be somewhat specialized and advanced but are not out of place.

Eight specific diseases caused by phycomycetes are described on the 45 pages in Chapter 8, including no bibliography or references. The diseases presented are caused by parasites covering the full range of variation of the fungi generally thought to be properly included in the group indicated and the authors are to be commended on their selections.

There are 10 diseases of plants caused by parasites described on 47 pages in Chapter 9, under the title of Diseases Caused by Bacteria. Mycological principles have been cut to the bone in relation to the organisms included in this chapter. The explanation of why a bacterial pathogen may have 2 or more scientific names is presented, but is difficult to understand and certainly unsatisfactory to a beginner. The diseases discussed are fairly well selected from a geographical viewpoint and also from the viewpoint of variable sources of inoculum and methods of dissemination.

The 10th chapter, covering 44 pages, deals with "virus-diseases" of plants. An introduction and historical review of the subject and its development, economic impor-

tance, symptoms, causal agent, properties of viruses, environmental, physical and chemical influences upon them, their movement, multiplication, vectors, dissemination, and control occupy the greater part of the chapter. The remainder deals with 4 well-selected plant diseases in which general statements made concerning "virus-diseases" are specifically applied.

The ascomycetes and 11 diseases caused by them on fruits, cereals, vegetables, field crops, and trees are dealt with in Chapter 11 and occupy 58 pages of concentrated, informative, and descriptive material. The selection of diseases and causal parasites is very representative with this group. The range of hosts affected is fair and proportional. Certain substitutions might be suggested, but the improvement would be only for limited geographical locations. The information presented is readily adaptable if necessary, but in most instances deals with the most economically important diseases, which, without saying, are also those upon which the most research has been done and about which most is known.

The imperfect fungi and diseases caused by 5 of them follow in the next 33 pages, constituting Chapter 12 of this book. The authors state that, "the imperfects induce more local and general necrosis . . . than any other group of symptoms," and, "most of the imperfects attack the aerial parts of plants"; yet, in their selection of 5 representative diseases, we find 3 of them caused by root infections of the host in the soil with similar secondary symptoms. The pathogenicity of a certain parasite was demonstrated "in 1908" and upon turning the page, lest the student forget, he is reminded again of the same fact. Likewise it is stated that a parasite forms "mats 2 to 12 inches in diameter," and a page or two later this information is repeated; again we find a "fungus carried on the seed," and less than 10 lines later, "the seed carries the organism." Why the losses caused by the cowpea root-knot organism should be added to the losses caused by the cotton wilt organism for quotation requires further explanation. These fungi, so frequently encountered, so variable in forms, so difficult for the student, and so inviting to the investigator apparently have not received the careful attention they in their own right deserve.

The basidiomycetes, divided under the 3 headings, Smuts, Rusts, and Wood and Root Rots are treated within the 85 pages of Chapter 13. The smuts are represented by 5 parasitic species in 3 genera, the rusts by 5 species in 3 genera and the wood and root rots by 3 parasitic organisms.

The treatment of the group, as a whole, is probably the best in the book and shows that first-hand acquaintance produces superior knowledge and more unified, complete and convincing description. The host-relation diagrams are particularly of interest and value in showing clearly to the student the complicated life cycle of these parasites. The illustrations are usually good. Names of spore forms are uniformly used, except where "basidiospore" appears in place of "sporidia." The general descriptive matter is definitely condensed and inclusive except, for instance, where the word "flower" appears 3 times in a 24-word sentence.

Diseases caused by seed plants and nematodes are presented in the next two chapters on 23 pages. The parasites and host range indicated are rather inclusive but severely condensed, probably because of the lack of their importance economically or because of the scarcity of these diseases in the central plains section of the country.

The final chapter deals with nonparasitic agents in relation to plant disease and it is indeed meager. The authors might have made a more variable selection for presentation. Four diseases are described, 3 of which are on apple, the fourth being a deficiency disease. A fair idea is obtained regarding the conditions that contribute toward their development and correction. Possibly this chapter could have been combined with Chapter 6, entitled "The influence of environment on plant disease."

The book is up-to-date and contains an accumulation of heretofore nonsummarized published data that gives it a definite, fact-containing, valuation seldom equalled. The use of words in this text is of special, atmosphere-giving interest and will be noticeable to all who read it. A few selected at random are here presented: ameliorated, dendritic, dirt, ephemeral, fuzzy, infectivity, mycelial, rattled, smutty, stoppage, tilth and viruliferous. The arrangement of the groups of diseases caused by classes of fungi, the bacteria, viruses, etc., is somewhat irregular when compared with previously published texts in plant pathology; but if this sequence has proved better in practice then it is fully justified.

The lack of references offers no great obstacle for the student, but references might be desirable to teachers and others who wish to examine the original papers themselves.—GEORGE F. WEBER, University of Florida, Gainesville, Florida.

DEVRIES, LOUIS. *German-English Science Dictionary*. 473 p. \$3.00. McGraw-Hill Book Co., Inc. (New York). 1939.

The German-English Science Dictionary, compiled by members of the science faculty of Iowa State College under the editorship of Dr. DeVries, will be welcomed by

all who have to read scientific German in the original and who often have only a passing knowledge of the language. The book contains not only scientific terms but also a wide selection of common words, a fact that endeared Patterson's Chemical Dictionary to its many users. The book is well edited and, notwithstanding its size and variety of subject matter covered, is being sold at a price that should bring it within reach of any graduate student. The compilers succeeded well in bringing together terms found in the various German-English scientific dictionaries of earlier publication date.

It is regrettable, however, that so few new words of the many encountered in recent scientific contributions have been added. For example, under "Vegetation," the editor lists only 7 compound nouns, while the reviewer has in his own footnotes to Biological Equivalents 21 additional words selected from current publications, each of which is not directly translatable but must be expressed by its proper equivalent. This is equally true of the word "Gesellschaft," under which at least 12 new words could be added to the listed 7. Such instances are numerous throughout the book.

The reviewer hopes that in future editions of this volume these omissions will be filled in.—ERNST ARTSCHWAGER.

FUNDAMENTAL STUDIES OF THE STRIPE SMUT OF GRASSES (*USTILAGO STRIAEFORMIS*) IN THE PACIFIC NORTHWEST¹

GEORGE W. FISCHER

(Accepted for publication August 30, 1939)

INTRODUCTION

Name of the Disease

The smut of various grasses, caused by *Ustilago striaeformis* (Westd.) Niessl., has been known by such common names as timothy smut, stripe smut, leaf smut, striped smut, and others, the last two names being more widely used. Davis (11) suggested that the disease should be known as striped smut of grasses, rather than timothy smut or leaf smut, because it has been shown that the organism is not confined to timothy, nor is its development restricted to the leaves of the plants infected, but is systemic and may sporulate in culms, leaf sheaths, and floral parts. The writer prefers the name stripe smut because it is more euphonious and is in some respects analogous to the name stripe rust, caused by *Puccinia glumarum* (Schmidt.) Eriks. and Henn.

Economic Importance

The effect of stripe smut on the host makes it a very destructive disease. The rupture of the long sori in the leaves results in the shredding and death of these organs, thereby materially weakening the plant and predisposing it to other sinister factors in its environment. This condition is accentuated by the fact that the leaf sheaths, culms, and floral parts are often attacked. Thus it is obvious that an infestation of stripe smut is a very important factor in the cultivation of grasses for seed, hay, pasturage, and even for lawns and putting greens. Clinton (6) reported substantial losses caused by stripe smut in fields of timothy (*Phleum pratense* L.) and redbtop (*Agrostis alba* L.) in Illinois. In one locality, where the latter grass was being grown for seed, Clinton found fully 30 per cent of the plants infected. The grower complained that the seed yield had been reduced to as low as 23 per cent of the normal yield. Osner (29), in making a survey of the extent of stripe smut of timothy in 9 counties of New York, found the disease more or less abundant in every field. In one field he reported over 50 per cent of the plants infected, and estimated that this represented about 30 per cent loss of hay. Osner further pointed out that if the timothy had been grown for seed the loss would have been greater. Pammel *et al.* (32) and Pammel (30, 31) reported considerable loss in the timothy fields on the Iowa State College farm. Numerous other reports could be cited,

¹ Grass-disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Soil Conservation Service, Section of Conservation Nurseries, and the Divisions of Plant Pathology and Agronomy of the Agricultural Experiment Station, State College of Washington.

which indicate that the disease is quite widespread from coast to coast. Davis (9) stated that stripe smut is principally confined to the humid central portion of the north temperate zone between the north parallels of 30 and 50 degrees.

Host Range

The host range of *Ustilago striaeformis* is quite extensive. Osner (29) listed 38 species and varieties of grasses, including both European and American hosts. Davis (9) stated that this species occurs on about 40 species of the Gramineae, Liro (28) listed over 30 species and varieties, while Seymour (35) in 1929 reported 20 species and varieties from North America. Considering that a number of species have been added since that time by various investigators, and that Seymour's report is not complete to 1929, it seemed probable that over 30 species and varieties of grasses serve as hosts for *U. striaeformis* in North America. In reviewing the available literature on the subject the following list of hosts for North America has been compiled, including a few new hosts established by inoculation experiments described in this paper. *Agropyron caninum* (L.) Beauv.,² (17),³ *A. cristatum* (L.) Gaertn. (17), *A. inerme* (Scribn. and Smith) Rydb. (17), *A. pauciflorum* (Schwein.) Hitchc. (17), *A. repens* (L.) Beauv. (34), *A. semicostatum* (Steud.) Nees,⁴ *A. smithii* Rydb.,⁴ *A. spicatum* (Pursh) Scribn. and Smith (17), *A. subsecundum* (Link) Hitchc.,⁴ *Agrostis alba* L. (35) (under *A. palustris* Huds.), *A. exarata* Trin. (35), *A. perennans* (Walt.) Tuckerm. (39), *A. palustris* Huds. (39), *A. tenuis* Sibth. (24) (as *A. alba vulgaris* Coss. and Dur.), *Anmophila arenaria* (L.) Link (7), *A. breviligulata* Fern. (35), *Beckmannia syzigachne* (Steud.) Fern. (34), *Bromus commutatus* Schrad. (39), *Calamagrostis canadensis* (Michx.) Beauv. (34), *C. pickeringii* A. Gray (35), *Dactylis glomerata* L. (35), *Elymus canadensis* L. (35), *E. canadensis* var. *robustus* (Scribn. and Smith) Mackenz. and Bush.,⁴ *E. glaucus* Buckl. (35), *E. sibiricus* L.,⁴ *E. virginicus* L. (35), *Festuca obtusa* Spreng. (7) (as *F. nutans*), *F. ovina* L. (35), *Holcus lanatus* L. (36), *Hordeum brevisubulatum* Trin.,⁴ *H. jubatum* L.,⁴ *H. jubatum* var. *caespitosum* (Scribn.) Hitchc.,⁴ *H. nodosum* L.,⁴ *Hystrix patula* Moench (25), *Lolium perenne* L. (36), *Phleum pratense* L. (35), *Poa annua* L. (35), *P. compressa* L. (35), *P. languida* Hitchc. (35) (as *P. debilis* Torr.), *P. pratensis* L. (35), *Sitanion hansenii* (Scribn.) J. G. Smith (18), *S. hystrix* (Nutt.) J. G. Smith (7) (as *S. longifolium* J. G. Smith).

REVIEW OF LITERATURE

From the contributions of previous investigators of *Ustilago striaeformis* and the smut of grasses induced by it, the following points in the life history and general biology of the pathogen may be summarized:

² Where possible Hitchcock (21) has been followed in attempting to determine the correct names for the grasses mentioned in this paper.

³ Numbers in parentheses refer to literature cited.

⁴ Hitherto unreported host, based upon data presented in this paper.

1. Spore germination studies have proved conclusively that the stripe-smut organism is a species of *Ustilago*, and should be called, therefore, *U. striaeformis* (6, 9, 29).
2. Spore germination is similar to that reported for *Ustilago nuda* (Jens.) Rostr., *U. tritici* (Pers.) Jens., *U. longissima* (Sow.) Tul., and *U. violacea* (Pers.) Roussel (9).
3. The smut spores of *Ustilago striaeformis* are resting spores, and an after-ripening period averaging 240 days in a damp atmosphere at about 20° C. (265 days in field) is prerequisite to successful germination (9).
4. After-ripened spores did not germinate on a variety of substrata unless sufficient moisture was present to float the spores or cover them with a film of solution (9).
5. Inoculations and cytological evidence have demonstrated that, as concerns stripe smut on timothy, seedling infection commonly occurs and is not originated by a dormant mycelium in the embryo (10).
6. At least 4 physiologic races have been differentiated, one restricted to each of the following hosts: *Phleum pratense*, *Agrostis alba*, *Poa pratensis* and *P. annua* (12). *Ustilago clintoniana* (12) and several other species (28) very closely allied to *U. striaeformis* may be only physiologic races of the latter species on other hosts.
7. To date all attempts to culture *Ustilago striaeformis* have been entirely unsuccessful (9, 10, 24).

MATERIALS AND METHODS

In this paper the contributions to the life history and pathogenicity of *Ustilago striaeformis* are based upon experiments with two collections of this smut fungus. One collection (herein designated as collection L-A) was made in August, 1936, from several plants in the same row of slender wheat-grass, *Agropyron pauciflorum*, in the Soil Conservation Nurseries, Pullman, Wash. The other collection (collection L-B) came from *Elymus glaucus*, July, 1937, also in the Soil Conservation Nurseries at Pullman.

All inoculations, made with sporidia or with spores, were made by a slight modification of the partial-vacuum method developed by Zade (38), and Haarring (19), and found by Allison (1) and others so effective in studies of smuts of oats and barley. The modification consisted in either planting the inoculated seeds very soon after processing without any drying, or, if the seeds had to be stored before planting, they were dried immediately after processing and were stored dry at room temperature. With either spores or sporidia it seemed to make little if any difference whether the seed was planted immediately after processing or stored dry at room temperature or at 5° C. The procedure followed by Haarring (18), whereby the processed seed was dried for 24 hours, incubated 20 hours in a moisture-saturated atmosphere, and then dried again before sowing, was not tried, since it entailed unnecessary handling of the seed. Allison, after the seed had been processed and then dried for 12 hours, tried two modifications of

Haarring's procedure: (1) after 24 hours' incubation in a water-saturated atmosphere the seed was sown while still moist; (2) seed was stored dry at 2° C. for 24 hours before sowing. He found that the two modified methods gave equally excellent results, and finally adopted the second because of its simplicity. In the present studies not even refrigerated storage was found necessary.

When chlamydospores were used for inoculum the desired suspension was obtained by placing infected leaves and other parts in a beaker or flask with a small amount of water and macerating the material so as to liberate the spores. Water was then added to bring the suspension to the proper dilution after which the bits of leaves and other debris were strained off. The seed was inoculated in small (15 cc.) vials each containing about 5 cc. of the suspension.

When monosporidial lines of opposite sex were used for inoculum the technique of preparing this was somewhat different from that used by Allison (1), who grew the lines in potato-dextrose solution and used the resulting suspension of sporidia in the nutrient solution for inoculum. In the present studies the sporidia were transferred from the agar to water or physiological salt solution, and the resulting suspension was used for inoculum. Copious production of fresh sporidia was obtained in 2 days by making smear cultures on plates of a special agar medium, described below, containing 8 per cent dextrose, 4 per cent malt extract, 1 per cent peptone, and 2 per cent agar. The monosporidial lines of opposite sex were thus cultured singly on the agar, but were brought together in essentially equal quantities in the inoculum suspension.

The monosporidial lines of *Ustilago striaeformis* were obtained by isolating single sporidia from germinating chlamydospores with the aid of a Chamber's micro-manipulator, following the method described by Hanna (18).

EXPERIMENTAL RESULTS

Life History Studies

1. *Spore Germination.* According to Davis (10) an after-ripening period of 240-250 days in a moist chamber at room temperature is prerequisite to spore germination in *Ustilago striaeformis*, which probably explains the very low germination percentages obtained by Osner (29), Clinton (6), Horsfall (23), Duggar (15), and others. However, some investigators apparently have not experienced such difficulty in germinating the spores of *U. striaeformis*. Liro (28) presents the results of a series of inoculation experiments with stripe smut on various grasses and makes no mention of any prerequisite to successful spore germination. He obtained infection merely by mixing dry seeds and spores together. However, it must be considered that Liro worked with collections of stripe smut from other grasses than those employed in Davis' experiments. On the other hand, Davis (8) stated, concerning collections of *U. striaeformis* on *Poa*

pratensis and *P. debilis* (*P. languida*), that fresh spores collected in May germinated readily in water. Pammel (33) mentioned that spores of *Tilletia striaeformis* (Westd.) Magnus (*U. striaeformis*) from *Phleum pratense* and *Poa pratensis* germinated readily. He did not state the medium.

In July, 1938, the writer received a collection of *Ustilago striaeformis* on *Poa pratensis* from H. W. Johnson, Bureau of Plant Industry, who had obtained it from the Forage Crops greenhouse at Arlington, Virginia, May 5, 1938. On July 25 the spores were tested for viability and, after about 36 hours, 85 per cent of them had germinated on potato-dextrose agar. Subsequent tests gave gradually lower percentages until, finally, the last test, on October 10, 1938, showed no germination at all. Thus, this collection of *Ustilago striaeformis* exhibited viable spores, with a high percentage of germination, merely after dry storage at room temperature.

A collection of *Ustilago striaeformis* on *Agropyron pauciflorum* (collection L-A mentioned above) made in August, 1936, was tested for spore viability as soon as it could be brought into the laboratory, but less than 5 per cent germination was observed. Another test late in September showed about 25 per cent viable spores. This collection was not tested further until the following spring, when less than 1 per cent germination was observed. Several of the infected plants were removed to the greenhouse in the fall of 1936, where they continued to produce smutted leaves, and later, culms and spikes. In March, 1937, a quantity of infected leaves was removed from these potted plants and samples of the spores were tested for viability. Even in cases where spores had been removed from fresh, unruptured sori, as high as 90 per cent germination was observed. It should be mentioned that the smutted leaves dried in the laboratory for a few days before the spores were tested. This spore material was used in spring inoculation experiments of 1937.

In July, 1937, *Ustilago striaeformis* was found on *Elymus glaucus* (collection L-B, mentioned above). At that time tests showed over 60 per cent spore germination. Subsequent tests every several weeks showed a gradual decrease in percentage of germination, but this was about 20 per cent, even as late as March 1, 1938, when monosporidial cultures were obtained from germinating chlamydospores. In a later test, May 26, 1938, there was less than 1 per cent germination.

Another collection, made in August, 1938, on *Elymus canadensis* in the Pullman, Wash., Soil Conservation Nurseries, was not tested for spore viability until March 13, 1939, when 50 per cent spore germination was obtained on malt extract-dextrose-peptone agar.

In contrast to the collections described in which good germination was secured without any after-ripening process, are several others from *Poa pratensis*, *Phleum pratense*, *Agrostis alba*, and *Holcus lanatus*, which did not germinate over 2 per cent over a period of 5 months.

All of the above-described collections had been stored in envelopes or packets under conditions of room temperature and humidity, the latter

usually running rather low in the dry-land areas of the Pacific Northwest. It is seen from the spore germination data of these collections that for some reason the after-ripening period, under moist conditions described by Davis (9) as prerequisite to spore germination, does not apply to all collections of *Ustilago striaeformis*, especially the race on *Agropyron* and *Elymus* in the Pacific Northwest. It is further seen that the collections of *U. striaeformis* on *Agropyron* and *Elymus* in the Pacific Northwest have a greater period of germinability than those studied by Davis (9). He found that approximately 75 days represented the period of germinability for the smut from timothy, orchard grass, and redtop, and 120 days for stripe smut on June grass (Kentucky blue grass). The writer's *Agropyron* and *Elymus* collections of *U. striaeformis* indicated a period of germinability of several months.

The process of germination in the collections of *Ustilago striaeformis* on *Agropyron pauciflorum* (col. L-A) and on *Elymus glaucus* (col. L-B) has been studied and appears to differ from that reported by Davis (9) for collections on *Agrostis alba*, *Dactylis glomerata*, *Phleum pratense*, and *Poa pratense*, and by Osner (29) for *U. striaeformis* on *Phleum pratense*. These investigators found the process to be the same in the collections from all 4 hosts. A single elongate unicellular promycelium was extruded from a rift in the spore wall. Sometimes 1 or 2 septa were formed. Short branches usually were developed on the promycelium and were considered to be lateral sporidia, although they were not abstricted. In a few cases Davis observed fusions between these "sporidia." No typical sporidia were observed, and Davis was of the opinion that they are seldom, if ever, produced. In no case did he obtain any saprophytic development from the germinating spores, even when they had been under observation for 60 days.

In the present studies it was observed that, after 24-36 hours under optimum conditions for germination, there emerges from the spore 2 or 3 thick germ tubes (Fig. 1, A and B). These rapidly elongate, and develop cross walls and branches (Fig. 1, C and D), and soon begin to bud off elliptical sporidia. By the third day numerous sporidia usually have been produced (Fig. 1, E) and accumulate in masses, so that around each germinated spore there develops a vigorous saprophytic colony of mycelium and sporidia. This type of germination occurs on potato-dextrose agar, or malt extract-dextrose-peptone agar, but on low nutrient agars or on plain agar sporidial formation is more or less inhibited. In such cases fusions between promycelial branches or segments soon occur and these give rise to long, vigorous, aerial infection hyphae. This is thought to be comparable to what Davis (9) observed and described as, "In three cases primary sporidia fused, the contents of one passed into the other and formed a conidium which developed a mycelial thread."

No observations by the writer of germinating spores of *Ustilago striaeformis* on a variety of media have yielded anything comparable to what Davis (9) observed and described as primary sporidia emerging directly

from the spore and budding. At other times, according to Davis, the "primary sporidia" remained within the spore. The sporidia observed in the present experiments were larger, more typical of sporidia in general, and

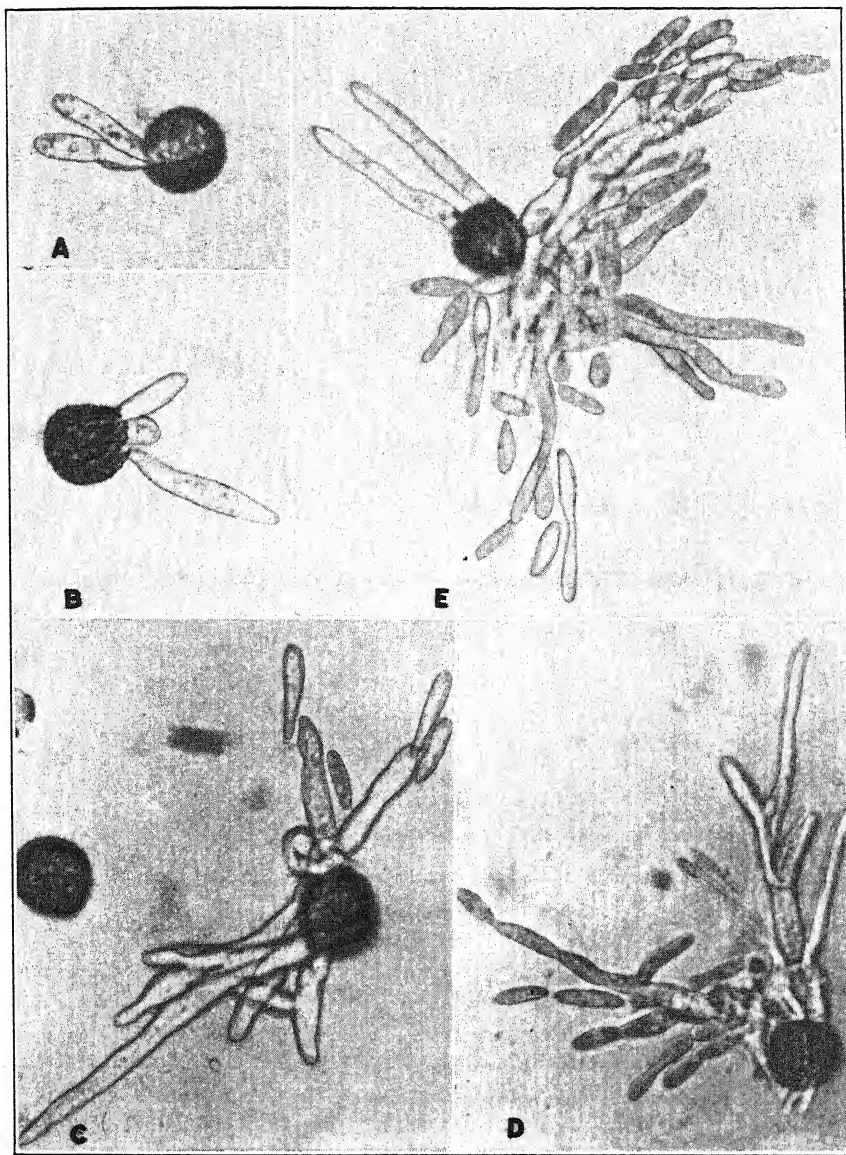


FIG. 1. Stages in spore germination in *Ustilago striaeformis*: A, B, after 36 hrs. on potato-dextrose agar; C, D, after 48 hrs.; E, after 60 hrs. Retouched enlargements of photomicrographs. \times approximately 1000.

were abstracted from the promycelial branches or segments. They were able to develop rapidly into large saprophytic colonies, an ability which heretofore has been entirely unknown in *U. striaeformis*.

2. *The Sexual Nature of the Sporidia.* Sometimes on nutrient agar, *in situ*, but especially when removed to plain, non-nutrient agar, the sporidia fuse in pairs with surprising rapidity. This fusion is accomplished when one (active) sporidium sends out a narrow fusion tube toward another (passive) sporidium nearby (Fig. 2, A) and fuses with that sporidium when contact has been made (Fig. 2, C-G). Occasionally, the juxtaposition of sporidia is such that 2 will try to fuse with the same sporidium, as seen in figure 2, B. Quite often 2 fused sporidia will be observed connected by a very long fusion tube, as shown in figure 2, H. Whether this represents the elongation of the fusion tube before fusion (in order to reach a sporidium some distance away) or an elongation after fusion, as Holton (22) observed in *U. avenae* and *U. levis*, has not been determined.

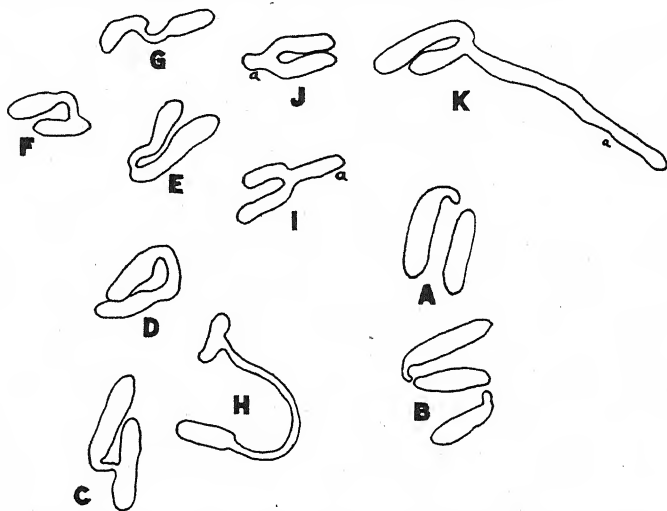


FIG. 2. Stages in the copulation of sporidia of *Ustilago striaeformis* and the subsequent development of infection hyphae. A and B. Development of fusion or copulation tube. C-H. Appearance of pairs of fused sporidia. I-K. Development of infection hypha, a. Drawn with the aid of the camera lucida. \times about 1100.

Shortly after fusion (2-4 hours), from one or the other of the fused pair, or from the fusion tube connecting them, a papilla appears (Fig. 2, I, a and J, a), which rapidly develops (Fig. 2, K, a) into a long vigorous aerial hypha. This is the "Suchfäden" described by Bauch (3, 4, 5). Within 12 hours after sporidia of opposite sex have been mixed on plain agar these "Suchfäden" or infection-hyphae have been produced in abundance and lend a cottony or whitish cast to the whole surface. Thus the well-known "Bauch test" (3, 4) for detecting sporidial lines of opposite sex is readily applicable to *Ustilago striaeformis*. In fact the fusion of sporidia and the subsequent production of "Suchfäden" or infection hyphae is very similar to that described for *Ustilago avenae* (23), *U. levis* (13, 23), *U. hordei* (1, 33), *U. bromivora* (Tul.) Fisch. de Waldh. (3), *U. violacea* (2, 27), *U. nigra* Tapke (*U. medians* Biedenk. (1)) and others.

The readiness with which the sporidia of the writer's cultures of *Ustilago striaeformis* fuse and develop infection hyphae indicated their sexual nature, and it was decided to investigate this further. Some preliminary studies of monosporidial lines will be reported here.

On March 1, 1938, from a small colony that had developed around a single germinated spore of collection L-B of *Ustilago striaeformis* (from *Elymus glaucus*), 15 sporidia were isolated and allowed to bud and develop into monosporidial colonies. Only 9 sporidia survived the isolating process, but these developed rapidly into colonies that were later transferred to test tubes of potato-dextrose agar. By March 10, 1938, each culture had grown to sufficient size to afford an abundance of material for inter-mating of the cultures. Accordingly, a small amount of each culture was mixed, in turn, with an equal amount of each of the other cultures on plain agar. Within 12 hours or even less it was possible to tell at a glance under the low power of the microscope where fusions had taken place. In fact, the whitish cast visible to the naked eye indicated where fusions and the production of infection hyphae had taken place. The results of these matings are shown in table 1.

TABLE 1.—Reactions of nine monosporidial cultures of *Ustilago striaeformis* (L-B)^a from the same germinating chlamydospore, when mated with each other on plain agar

Designation of cultures									
	11	12	13	14	15	16	17	18	19
11	— ^b	—	—	+	—	+	+	—	+
12		—	—	+	—	+	+	—	+
13			—	+	—	+	+	—	+
14				—	+	—	—	+	—
15					—	+	+	—	+
16						—	—	+	—
17							—	+	—
18								—	+
19									—

^a Collection L-B is from *Elymus glaucus*.

+ = abundant fusions and infection hyphae.

^b Legend: — = no action.

On the basis of the reactions shown in table 1 it is seen that of the 9 cultures, 5 were of 1 sex group and 4 of another. By arbitrarily designating the first culture (No. 11) as + the others are segregated for sex as follows:

	+	+	+	—	+	—	—	+	—
Culture No.	11	12	13	14	15	16	17	18	19

From these results it seems that only 2 sex groups are represented in *Ustilago striaeformis* and that these probably are segregated on a 1:1 basis.

3. "Hybridization" with Other *Ustilago* spp. Kniep (27) considered hybridization as having been effected between species of smut fungi when their sporidia had been made to fuse. He obtained fusions between the

sporidia of several *Ustilago* spp. but did not attempt to obtain F_1 chlamydospores from these combinations by inoculating the fused sporidia into a probable host. Dickinson (14) obtained infection (as evidenced by histological examination of inoculated oat seedlings) from combinations of sporidia of opposite sex of *U. levis* and *U. hordei*, but did not state that any sporulation occurred in the host. Since neither Kniep nor Dickinson obtained F_1 chlamydospores from their "hybrids," and since Allison (1) has shown that, at least in the barley smuts, soon after the fusion of two sporidia (of different as well as of same species) merely the dikaryophase is initiated by association of the two nuclei in one sporidium and later in the infection hypha, the fusing of sporidia cannot be considered true hybridization. It is fairly well established that in the smut fungi this dikaryophase persists throughout the development of the mycelium in the host plant up to the time of sporulation, and that the true diploid condition is initiated at the time of fusion of the two nuclei in the young spore. True hybridization occurs, then, when interspecific combinations of monosporidial lines of opposite sex result in sporulation on some host. Such hybrids have been produced between *U. avenae* and *U. levis* (22), *U. hordei* and *U. nigra* (1), *Sphacelotheca sorghi* (Link) Clint. and *S. cruenta* (Kühn) Potter (34), *S. sorghi* and *Sorosporium reilianum* (Kühn) McAlpine (37), and other species.

In the present studies 4 monosporidial lines of *Ustilago striaeformis*, L-B, (2 of each sex group) were mated with 12 pedigreed monosporidial lines of *U. bullata* (1 of each of 2 sex groups from 6 collections) on plain agar. The results are recorded in table 2.

From the data shown in table 2 it is readily seen that: (1) The sporidia of *Ustilago striaeformis* are highly compatible with those of *U. bullata*; (2) certain combinations are far more productive of infection hyphae than are others; and (3) in every case the greater number of infection hyphae involved the same sex group of *U. striaeformis*. Thus, it was cultures L-B 11 and 12 that produced the abundance of infection hyphae when combined with cultures of opposite sex of *U. bullata*, although the other cultures of *U. striaeformis*, L-B 17 and 19, entered just as readily into sporidial fusions with the cultures of opposite sex of *U. bullata*. This situation is somewhat similar to that described by Bauch (4) who discovered that *Sphacelotheca schweinfurthiana* (v. Thüm.) Sacc. exhibited 2 different types of reaction between the monosporidial lines of opposite sex; in one case ("W-Reaktion") no further development of the fused sporidia took place, whereas, in the other ("S-Reaktion"), binucleate infection hyphae developed from the fused sporidia in the normal manner. Bauch's results, however, were obtained from intraspecific mating experiments, whereas those here described apply to interspecific matings. Within either of the species concerned, *U. striaeformis* and *U. bullata*, such reactional differences have not been observed. These differences are thought by the writer to represent differences in the compatibility between monosporidial lines of opposite sex of 2 species

TABLE 2.—Reactions of 4 monosporidial cultures of *Ustilago striaeformis* with 12 pedigreed monosporidial cultures of *Ustilago bullata* when mixed on plain agar

Ustilago bullata	Pedigreed monosporidial cultures	<i>U. striaeformis</i> , L-B, ^b Monosporidial cultures			
		11	12	17	19
<i>U. bullata</i>	N-A 111	++++ ^a	+++	—	—
“	“ 112	—	—	+	+
“	N-J 51	—	—	+	+
“	“ 52	+++	+++	—	—
“	R-A 71	—	—	+	+
“	“ 72	+++	+++	—	—
“	R-G 51	—	—	++	++
“	“ 53	+++	+++	—	—
“	M-C 121	—	—	+	+
“	“ 123	+++	+++	—	—
“	M-L 61	+++	+++	—	—
“	“ 63	—	—	+	+

^aLegend: +++ = abundance of both fusions and infection hyphae.

+++ = “ “ fusions but less of infection hyphae.

++ = “ “ , with few infection hyphae.

+ = “ “ “ , very few or no infection hyphae.

— = neither fusions nor infection hyphae.

^b *U. striaeformis* L-B = collection from *Elymus glaucus*; *U. bullata* N-A = collection from *Agropyron pauciflorum*, N-J = col. from *E. canadensis*; R-A = *ibid.* from *Hordeum nodosum*; R-G = *ibid.* from *Sitanion jubatum* J. G. Smith; M-C = *ibid.* from *Bromus marginatus* Nees.; M-L = *ibid.* from *Bromus anomalus* Rupr. M-C and M-L have earlier been known as *U. bromivora* (Tul.) Fisch. von Waldh. (16). The arabic numerals appended to the collection symbols indicate the pedigree of the monosporidial cultures. In every case the last digit indicates the origin of the culture with reference to the position, on the promycelium, of the isolated sporidium from which the culture originated. The remaining digit or digits refer to the number given the chlamydospore from whose promycelium the sporidia were isolated. The numbering of the chlamydospores always begins with the digit 5, so as to avoid confusion with sporidium numbers that ordinarily range from 1 to 4. Arbitrarily, the sporidium from the distal cell of the promycelium is designated as No. 1, and the numbering proceeds toward the spore. (Thus, monosporidial culture M-C 123 arose from the third sporidium on the promycelium of chlamydospore No. 12). This method of indicating pedigree could not be applied to *U. striaeformis* because of the indefinite position of the sporidia on the branched promycelium.

of smut fungi, due to the association, in the copulating sporidia, of 2 nuclei representing the 2 different species. In one case the 2 nuclei are compatible and an infection hypha results; in the other case the association is incompatible and usually no further development takes place. However, this fundamental difference between the nuclei must be independent of sex potential; otherwise, presumably, sporidia of opposite sex, but having incompatible nuclei, would not even fuse.

The profuse development of infection hyphae resulting from sporidial fusions between certain monosporidial lines of opposite sex of *Ustilago striaeformis* and *U. bullata*, as compared with the absence of such infection hyphae in other combinations of opposite sex of the same species, suggests that the former type might be expected to represent sufficient compatibility to result in the development of mycelium and F₁ chlamydospores when applied to some common host. Likewise, the sporidial fusions wherein very few or no infection hyphae result might not be expected to result in such infection and spore production. Experiments are in progress to test out

these possibilities on *Agropyron pauciflorum*, *Elymus canadensis* and *E. sibiricus*, which are common hosts for both species of smut fungi.

Cultures L-B 11, 12, 17, and 19 of *Ustilago striaeformis* also were mated with monosporidial cultures of *Ustilago nigra* and *U. hordei*, but, although in some cases rather numerous sporidial fusions resulted, a very low degree of compatibility was indicated as compared with the combinations with *U. bullata*.

4. *Behavior of Ustilago striaeformis in Culture on Agar Media.* Although earlier investigators have reported that *Ustilago striaeformis* is incapable of development on artificial media, the writer has found that the race of this species, which occurs on *Agropyron* and *Elymus* in the Northwest, is one of the most easily cultured and most rapidly growing smut fungi in his experience.

Shortly after obtaining the 9 monosporidial cultures of opposite sex, as described above, it was decided to try a culture of each of the 2 sex groups on different agar media. For a preliminary experiment 4 agar media were finally selected: (1) raisin agar, containing an infusion from 100 grams raisins per liter, with .1 per cent peptone added; (2) potato-dextrose agar, containing 200 grams potatoes and 20 grams dextrose per liter; (3) "P. D. A. +," a modification of potato-dextrose agar, containing 1.5 per cent malt extract and .1 per cent peptone per liter; and (4) pea agar, containing an infusion from 300 grams dried peas, 2 per cent dextrose, 1.5 per cent malt extract, and .1 per cent peptone per liter. Duplicate 50 mm. Petri dishes of these media were inoculated and incubated at room temperature (20-22° C.) for four weeks. The growth response of the two cultures is illustrated in figure 3. The growth on the raisin agar was rather weak, but was good on the other three media. With both sexes the best growth was obtained on the modified potato-dextrose agar.

Since the agar containing potatoes, dextrose, malt extract, and peptone proved to be the best in the above experiment, an attempt was made to determine the optimum proportion of these ingredients. Accordingly, 25 different combinations of these, all in 2 per cent agar, were prepared and poured into 50 mm. Petri dishes (Table 3), and represent only third of the possible combinations at the gradations shown. In conjunction with a similar experiment with other grass smut fungi, these plates were inoculated in duplicate with one monosporidial culture of *Ustilago striaeformis* (L-B 17). They were incubated at room temperature for 2 weeks, at the end of which time the diameter in mm. of each colony was recorded. The data are presented in table 3 and the growth response is shown in figure 4.

The results of the above experiment indicate a rather wide tolerance in *Ustilago striaeformis* of high concentrations of the nutrients used. It is surprising that growth was obtained on agar No. 25, containing 40 per cent dextrose, as is the tolerance to 35 per cent dextrose in agars No. 20 and 24. One of the agars resulting in the largest colonies is No. 15, containing 25 per cent dextrose; but, on the other hand, some of the other agars just as

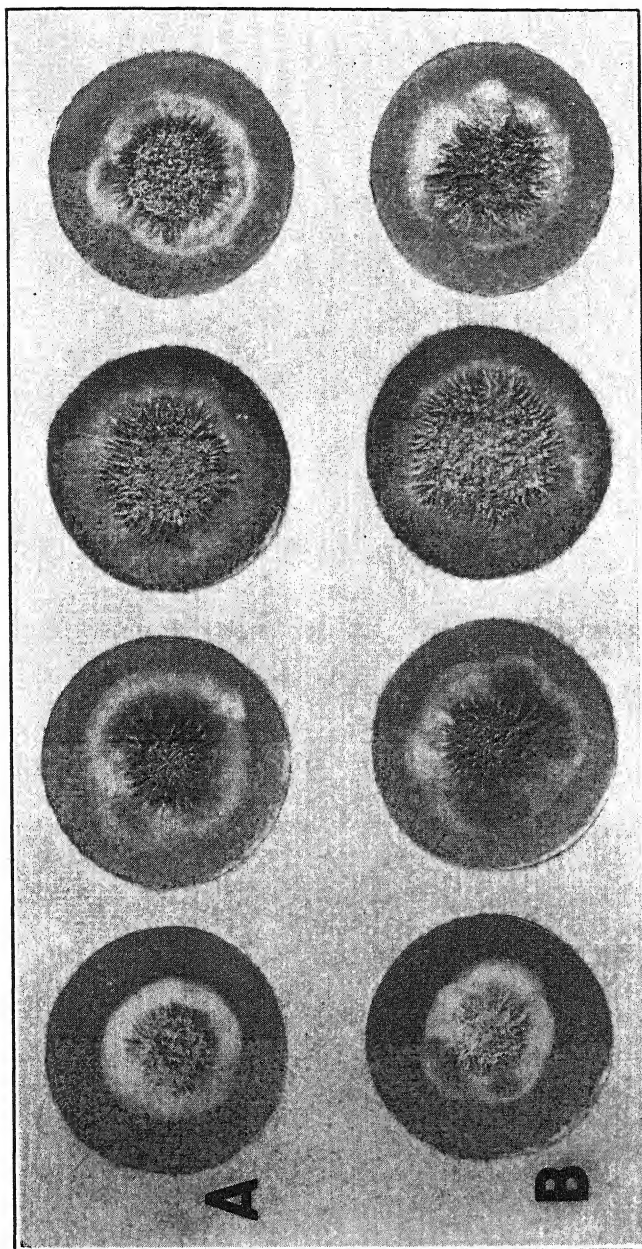


FIG. 3. Growth of *Ustilago striaeformis* (two cultures of opposite sex) on agar media. A. Culture L-B 11 on (left to right) raisin agar, potato-dextrose agar, malt-extract agar, and pea-infusion agar. B. Culture L-B 19 on the same media. Four-weeks growth in 50-mm. Petri dishes, at room temperature.

TABLE 3.—Summary of the size of colonies produced on 25 combinations of potato decoction, dextrose, malt extract, and peptone in 2 per cent agar, by *Ustilago striaeformis* (L-B). Two weeks' growth at room temperature

Agar	Composition of the agars				L-B 17
	Dextrose	Potatoes	Malt extract	Peptone	Diameter of colony
No.	per cent	grams	per cent	per cent	mm
1	0	400	20	0.2	15
2	1	300	15	0.3	15
3	2	200	10	0.5	24
4	4	100	6	0.75	24
5	8	0	4	1.00	26
6	1	500	15	0.15	16
7	2	400	10	0.2	13
8	4	300	6	0.3	15
9	8	200	4	0.5	16
10	15	100	2	0.75	20
11	2	600	10	0.1	10
12	4	500	6	0.15	19
13	8	400	4	0.2	23
14	15	300	2	0.3	14
15	25	200	1	0.5	27
16	4	700	6	0.05	18
17	8	600	4	0.1	27
18	15	500	2	0.15	24
19	25	400	1	0.2	20
20	35	300	0.5	0.3	16
21	8	800	4	0.0	27
22	15	700	2	0.05	25
23	25	600	1	0.1	20
24	35	500	0.5	0.15	13
25	40	400	0	0.2	6

good, at least from the standpoint of the size of colony produced, numbers 3, 4, 5, 17, 18, 21, 22, contain from 2–15 per cent dextrose.

It would appear from the data that different amounts of peptone and potato cause less difference in growth response, from the standpoint of size of colony, than do the dextrose and malt extract. However, the experiment indicates that, while no one combination of the 25 tested is undoubtedly optimum, certain proportions of potato, dextrose, malt extract, and peptone are decidedly better than others for the production of vigorous colonies of a good sporidial consistency. In general, agar No. 5, containing 8 per cent dextrose, 4 per cent malt extract, and 1 per cent peptone, represents one of the best if not the best combination tried. This formula is being used at the present time for all of the writer's cultures of *Ustilago striaeformis*, as well as for other smut fungi.

Inoculation Experiments

Inoculation experiments with *Ustilago striaeformis* have been performed by Davis (10, 11, 12), Osner (29), and Liro (28), although the work by Liro was done under other species (biologic species) names. Davis inoculated seedlings of *Agrostis alba*, *Dactylis glomerata*, *Phleum pratense*, and *Poa* spp. Osner inoculated *Phleum pratense* and obtained very inconclusive

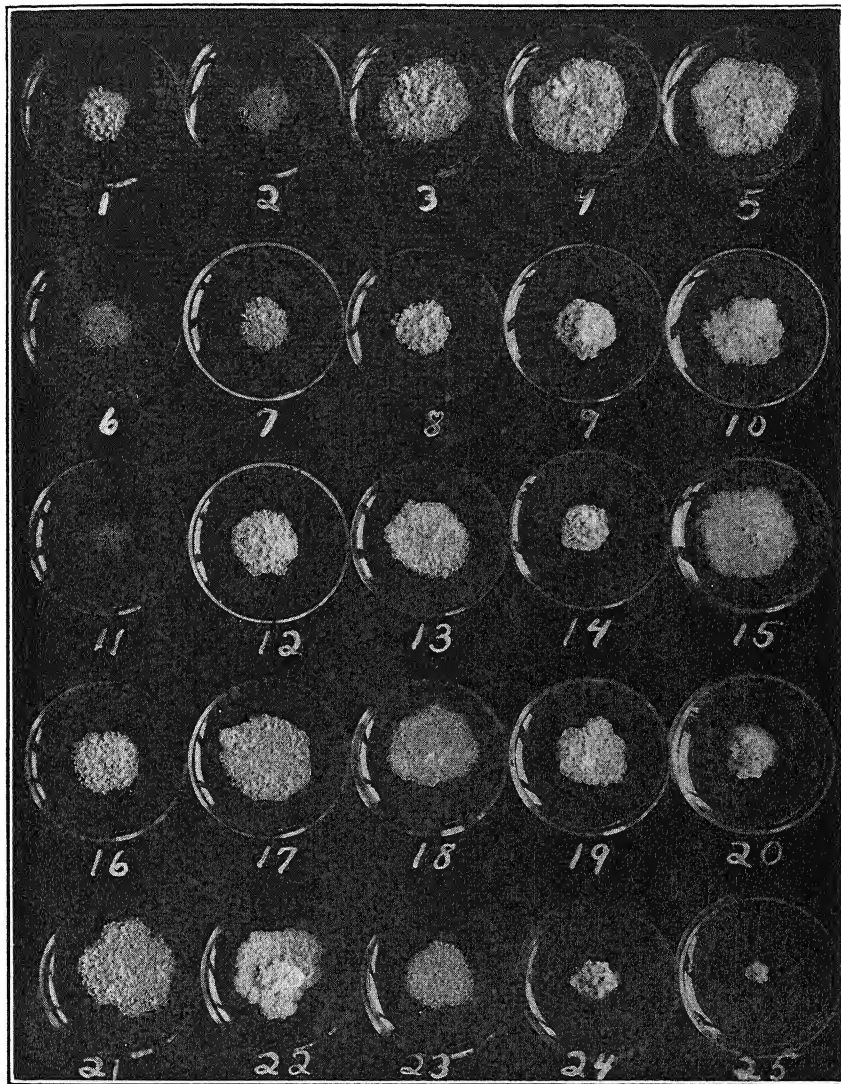


FIG. 4. Growth of *Ustilago striaeformis* on 25 different combinations of potato decoction, dextrose, malt extract, and peptone in 2 per cent agar. Compare with Table 3. Four-week growth in 50-mm. Petri dishes.

results, it being obvious that his seed already carried the fungus. Davis secured no higher than 60 per cent infection of timothy seedlings by seedling inoculation, and, even then, the hulls had been removed from the seed. By planting timothy seed in soil artificially contaminated with smut spores of *U. striaeformis*, he obtained as high as 72 per cent infection. His method of inoculation was quite laborious: the inoculum had to be "after-ripened" under specified conditions of time, temperature, and moisture; the spores would not germinate unless covered with a film of water; and the seeds were (except by soil inoculation) germinated and hulled before inoculation.

Osner (31) believed he had sufficient evidence to prove that blossom infection obtained in *Ustilago striaeformis*, but Davis (11) definitely disproved this. Exhaustive blossom-inoculation experiments gave negative results.

Liro (28) obtained satisfactory results merely by mixing seed and spores together (presumably dry).

1. *Field Experiments in 1937.* Inoculation experiments with *Ustilago striaeformis* by the writer indicate that such studies with this race on *Agropyron*, *Elymus*, etc., in the Northwest involved considerably less complication and trouble than was experienced by Davis in his studies of races on *Agrostis*, *Phleum*, *Poa*, etc. Noting that some plants of *Agropyron pauciflorum* in the greenhouse (transplanted from the field where they had been found infected with stripe smut) were producing sori in abundance somewhat prior to the time of the annual spring (1937) seeding and inoculation experiments, it seemed that here might be some inoculum for such experiments with stripe smut. Spores smeared over potato-dextrose agar germinated about 90 per cent. Considerable inoculum was collected from these greenhouse plants over a period of about a month and very little reduction in percentage of germination was observed during this time. The inoculum was prepared in the form of a spore suspension as already described along with the method of inoculation, under "Materials and Methods."

As a preliminary experiment, 50 seeds of each of the grasses listed in table 4 were inoculated, allowed to dry enough to permit handling, and then sown in 5-foot nursery rows. Within a few weeks after seeding, some of the grasses (*Agropyron subsecundum*, *A. pauciflorum*, et al.) began to show the first symptoms of infection, and others followed sooner or later. The final data from this experiment are present in table 4.

The data recorded in table 4, while based on too few plants per row to be very reliable, did nevertheless indicate the susceptibility of various species of *Agropyron*, *Elymus*, and *Hordeum* to this collection of *Ustilago striaeformis* from *Agropyron pauciflorum*.

2. *Field Experiments in 1938.* In March, 1938, after monosporidial cultures of opposite sex of *Ustilago striaeformis* had been obtained, it was decided to try these as inoculum. Allison (1) recently had reported great success in the use of suspensions of sporidia of opposite sex as inoculum in his studies of *U. hordei* and *U. nigra*. It was thought that if sporidial cultures of the stripe-smut organism could be used for inoculum, one need not be concerned with having a supply of viable chlamydospores for such purposes.

As a preliminary test of the efficacy of suspensions of sporidia of opposite sex as inoculum, approximately 200 seeds of *Agropyron subsecundum* (Acc. No. 13), shown in 1937 to be highly susceptible, were so inoculated, using the partial vacuum method, and sown in rows in the greenhouse. The seed was not allowed to dry before planting. Within 4 weeks after planting the first sori appeared; at the end of 6 weeks they were abundant. Of 164

TABLE 4.—Reaction of *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum* and *Sitanion* spp. to *Ustilago striaeformis* from *Agropyron pauciflorum*.

Grass species	Writer's Acc. No.	No. plants	No. smutted	Percentage infection
<i>Agropyron caninum</i>	282	9	9	100
“ <i>cristatum</i>	290	1	1	100
“ “	304	— ^a	0	0
“ <i>elongatum</i>	299	—	0	0
“ <i>inerme</i>	268	1	1	100
“ “	270	5	1	20
“ <i>pauciflorum</i>	249	—	0	0
“ “	288	5	1	20
“ “	251	4	2	50
“ “	324	7	7	100
“ <i>pungens</i>	298	—	0	0
“ <i>sibiricum</i>	291	—	0	0
“ <i>smithii</i>	285	—	0	0
“ <i>subsecundum</i> ...	13	6	6	100
“ “	309	—	0	0
“ <i>spicatum</i>	264	—	0	0
“ “	265	3	1	33
“ <i>repens</i>	297	—	0	0
“ <i>trichophorum</i> ...	296	—	0	0
<i>Bromus ciliatus</i>	247	—	0	0
“ <i>marginatus</i>	48	—	0	0
“ <i>tectorum</i>	165	—	0	0
<i>Elymus canadensis</i>	128	—	0	0
“ <i>glaucus</i>	3	4	4	100
“ “	5	3	1	33
“ <i>striatus</i>	124	—	0	0
“ <i>virginicus</i>	131-35	0	0	0
<i>Festuca elatior</i>	191	—	0	0
<i>Hordeum brevisubulatum</i> ...	302	5	1	20
<i>Sitanion hystrix</i>	300	—	0	0

^a — = number of plants was not recorded in rows showing no smut.

plants in the rows, 149, or about 90 per cent, showed smut typically and unmistakably. A check row of the same grass contained no smut. This preliminary test indicated that sporidia should be reliable inoculum for field plantings.

In the spring of 1938 (Apr. 16-20) the following species were inoculated with sporidia of opposite sex and seeded in 5½-ft. nursery rows: *Agropyron caninum*, *A. ciliare*, *A. cristatum*, *A. desertorum*, *A. elongatum* (Host) Beauv., *A. inerme*, *A. pauciflorum* (34 collections and selections), *A. pungens*, *A. sibiricum*, *A. smithii*, *A. spicatum*, *A. semicostatum*, *A. subsecundum*, *A. trichophorum* (Link) Richt.; *Agrostis alba*; *Arrhenatherum elatius* (L.) Mert. and Koch; *Bromus ciliatus* L., *B. marginatus* Nees; *Dactylis glomerata*, *Elymus canadensis*, *E. sibiricus*, *E. villosus* Muhl., *E. virginicus* var. *australis* (Scribn. and Ball) Hitchc.; *Holcus lanatus*; *Hordeum brevisubulatum*, *H. bulbosum* L., *H. gussoneanum* Parl., *H. jubatum*, *H. jubatum* var. *caespitosum*, *H. murinum* L.; *Lolium perenne*; *Phleum pratense*; *Poa pratensis*; *Sitanion hystrix*.

In general, good stands resulted from these inoculated seeds. In some rows smut began to appear as early as June 1. Infection counts were de-

layed until about the middle of July when it was noticed that in some of the rows the plants were succumbing to the infection. Row by row, the plants were uprooted and the total number and the number showing smut in each row were recorded (Table 5). The data concerning the 34 selections and

TABLE 5.—Reaction of *Agropyron*, *Elymus* and *Hordeum* spp. to a collection L-B of *Ustilago striaeformis* from *Elymus glaucus*

Species	Writer's Acc. No.	S.C.N. No.	No. plants	No. smutted	Percent- age smut
<i>Agropyron cristatum</i>	29		15	2	13
“ “	36		72	11	15
“ “	290	W. 2413	138	25	18
“ <i>semicostatum</i>	377	W. 2903	75	11	15
“ <i>subsecundum</i>	309		48	20	42
“ “	12		37	5	14
“ “	13		30	18	60
“ sp.	310		112	2	
<i>Elymus canadensis</i>	341	W. 2389	79	61	77
“ <i>sibiricus</i>	335	W. 214	79	51	65
“ “	338	W. 225	201	181	90
<i>Hordeum brevisubulatum</i>	302	W. 303	85	8	9
“ <i>jubatum</i> var. <i>caespitosum</i>	334		13	12	92
“ <i>jubatum</i>	15		33	17	52

collections of slender wheatgrass will be presented and discussed below, under “Varietal reaction of selections and collections of slender wheatgrass to *Ustilago striaeformis*.”

Of the 33 species and varieties inoculated only 7 showed smut. These are listed in table 5. On the basis of these results *Agropyron pauciflorum*, *A. subsecundum*, *Elymus canadensis*, *E. sibiricus*, *Hordeum jubatum*, and *H. jubatum* var. *caespitosum* are highly susceptible to this collection (L-B) of *Ustilago striaeformis* from *Elymus glaucus*. The absence of even a trace of stripe smut on any of the accessions of redbtop, timothy, orchard grass, Kentucky blue grass, and velvet grass indicates further proof that the stripe smut on *Agropyron* and *Elymus* in the Northwest is a race very distinct from the races studied by Davis (11, 12) and Osner (29).

3. *Greenhouse Experiments.* The successful inoculation of *Agropyron subsecundum* with *Ustilago striaeformis* in the greenhouse, as described above, suggested that possibly studies of the stripe smut of grasses could be carried out in the greenhouse, since it would not be necessary for the grasses to come to maturity, as it is with inoculation experiments with most smuts. If good infection could be obtained from sporidia or spores and a reading obtained within 6 weeks, as the preliminary inoculation of *A. subsecundum* indicated, then greenhouse studies of stripe smut could be pursued with almost as much ease as are those that concern the rusts of cereals and grasses.

On Jan. 21, 1939, 40–50 seeds of each of the following grasses were inoculated with (1) chlamydospores of *Ustilago striaeformis* collection L-A (from *Agropyron pauciflorum*), (2) chlamydospores of collection L-B (from

Elymus glaucus), and (3) suspensions of sporidia from monosporidial cultures of opposite sex of collection L-B: *Agropyron caninum*, *A. ciliare* Franch., *A. cristatum*, *A. desertorum* (Fisch.) Schult., *A. elongatum*, *A. inerme*, *A. repens*, *A. semicostatum*, *A. sibiricum* (Willd.) Beauv., *A. smithii*, *A. spicatum*, *A. subsecundum*, *A. trichophorum*; *Agrostis alba*; *Arrhenatherum elatius*; *Bromus brachystachys*, *B. brizaeformis* Fisch. and Mey., *B. erectus* Huds., *B. hordeaceus* L., *B. inermis* Leyss., *B. japonicus* Thunb., *B. mollis* L., *B. tectorum* L.; *Dactylis glomerata*; *Elymus canadensis*, *E. canadensis* var. *robustus*, *E. glaucus*, *E. sibiricus*, *E. villosus*, *E. virginicus*, *E. virginicus* var. *australis*; *Festuca idahoensis* Elmer; *Holcus lanatus*; *Hordeum gussoneanum*, *H. jubatum*, *H. jubatum* var. *caespitosum*, *H. brevissubulatum*, *H. nodosum*; *Lolium perenne*; *Phleum pratense*; *Poa pratensis*; *Sitanion hansenii*.

On Feb. 24, 1939, the first sori were noticed, although it is probable that by careful searching they might have been found before that date. On March 25 data were taken, recording the total number of plants and the number showing smut for each inoculation.

Of the 42 species and varieties inoculated, 11 showed stripe smut in varying percentages. These are listed in table 6, which also contains infection percentages indicating the relative efficacy of spores and sporidia as inoculum.

TABLE 6.—Reaction of species of *Agropyron*, *Elymus*, *Hordeum*, and *Sitanion* when inoculated with spores and sporidia of *Ustilago striaeformis* in the greenhouse

Species	Acc. No.	Percentage of infection ^a			
		Check	Col. L-A (spores)	Col. L-B (spores)	Col. L-B (sporidia)
<i>Agropyron caninum</i>	139	0	34	91	86
“ <i>cristatum</i>	195	0	0	5	0
“ “	210	0	0	0	10
“ <i>inerme</i>	268	0	30	27	38
“ <i>smithii</i>	308	0	11	0	15
“ <i>subsecundum</i>	309	0	0	2	0
“ “	138	0	29	5	11
“ “	12	0	44	10	0
“ “	13	0	11	34	56
<i>Elymus canadensis</i>	341	0	33	17	25
“ “ var. <i>robustus</i> ..	126	0	0	28	13
“ <i>glaucus</i>	127	0	34	0	0
“ “	342	0	29	6	29
“ <i>sibiricus</i>	335	0	0	4	17
“ “	338	0	33	44	41
<i>Hordeum nodosum</i>	169	0	7	0	0
“ “	189	0	0	2	9
<i>Sitanion hansenii</i>	392	0	50	0	0
Average		0	19	15	19

^a Based on plant counts.

The results of this inoculation experiment indicate that, in general, sporidia of opposite sex are just as effective for inoculum in greenhouse

studies of this race of *Ustilago striaeformis* as are spores. Considering the ease with which *U. striaeformis* may be cultured and the rapid growth of the cultures it seems that sporidia might well be used entirely for inoculation experiments, and the investigator would not have to be concerned with the necessity of maintaining a supply of viable chlamydospores.

On the basis of the results of this experiment it cannot be determined definitely whether collection L-A, from *Agropyron pauciflorum*, is pathogenically different from collection L-B, from *Elymus glaucus*.

A comparison of the data in table 5 with those in table 6 indicates to some extent that infection percentages obtained in the field were generally higher than those obtained in the greenhouse, even with the same grasses. Further experiments are necessary definitely to prove or disprove this. The smut sori begin to appear in inoculated grasses in the greenhouse as soon as or sooner than in the field, and data on a series of inoculations can be taken within 2 months after planting. It is thought that eventually inoculations of grasses with *Ustilago striaeformis* can be as reliably conducted in the greenhouse as can those with the various rusts.

Varietal Reaction of Selections and Collections of Slender Wheatgrass to *Ustilago striaeformis*

Considering the effect of stripe smut on its hosts, it is the opinion of the writer that this disease has the possibilities of becoming one of the most serious of slender wheatgrass and certain other grasses in the Northwest. As has been true in the research programs concerning other plant diseases, the search for resistant or immune strains of the host plants should be included in practical studies of the stripe smut of grasses.

Since the writer had several collections and selections of slender wheatgrass and since many others were available through the courtesy of cooperating agencies, it was decided to test these for relative susceptibility to *Ustilago striaeformis*. Thirty-four strains of slender wheatgrass were inoculated with collection L-B (from *Elymus glaucus*) of *U. striaeformis*. The seed was inoculated with an aqueous suspension of sporidia of opposite sex, and planted in nursery rows in the spring of 1938. Although the smut began to appear in a few weeks after seeding, the taking of data was deferred until about the middle of July, when it was noticed that many of the infected plants were dying due to inability to withstand drouth because of the shredded condition of the leaves, produced by the stripe-smut infection. The plants in each row were uprooted and data taken on a plant-count basis (Table 7).

As seen from table 7, there is surprisingly little resistance in the 34 selections and collections of slender wheatgrass inoculated with stripe smut. Only 6 selections appeared promising as resistant or immune stock: Acc. Nos. 74, 137, 249, 250, 252, 259. These results indicate that slender wheatgrass, as a species, is quite susceptible to stripe smut, and, such being the case, attention should be given in the grass improvement program to selections resistant to or immune from this disease.

TABLE 7.—*Varietal reaction of selections and collections of slender wheatgrass to Ustilago striaeformis from Elymus glaucus*

Writer's Acc. No.	Other No.	No. plants	No. smutted	Percentage smut
6		20	11	55.0
56	Sel. from Wn. 279 ^b	84	58	69.0
57		111	45	40.5
58	Wn. 251	38	18	47.3
59	Wn. 241	73	41	58.0
60	Wn. 239	38	12	31.6
61	Sel. from Wn. 279	60	24	40.0
62	Wn. 249	296	90	30.4
63	Wn. 242	76	23	30.2
64	Sel. from Wn. 279	211	33	15.6
65	do	114	41	35.9
66	do	32	17	53.1
67	do	85	77	90.5
68	do	172	59	34.3
69	Wn. 253	168	86	51.1
70	Wn. 512	87	31	35.6
71		156	52	33.3
72	Wn. 435	57	36	63.1
73	Wn. 434	99	65	65.6
74	Wn. 472	187	14	7.4
75	Sel. from Wn. 279	103	46	44.6
83		106	43	40.5
84		100	35	35.0
137 ^a	0	0.0
249	Var. "Grazier" W. 3123 ^c	104	2	1.9
250	Var. "Fyra" W. 3218	0	0.0
251	W. 2712	205	56	26.3
252	W. 934	13	1	7.7
253	W. 591	70	29	41.4
254	Var. "Mecca" W. 3124	217	72	34.1
259	W. 879	0	0.0
288		138	53	38.4
306		150	44	29.3
324		59	29	49.1

^a Number of plants was not determined in rows showing no smut.^b Numbers preceded by Wn. are of the Wash. Agr. Exp. Stat.^c " " " " " W " " " " Pullman Unit of the Soil Conservation Service, Section of Nurseries.

The Possibility of Seed Carriage in the Life History of *Ustilago striaeformis*

Davis (10) has shown that as far as stripe smut in timothy is concerned, infection occurs from after-ripened spores in the soil. He showed that infection seldom, if ever, results from seed-borne spores or mycelium. Of 208 plants resulting from seed taken from infected plants, only one showed smut and, as Davis (10) points out, it was in one of the uncontrolled plots. He thus showed that seeds borne on infected culms do not produce infected seed, either as a result of penetration of the mycelium of the host plant into the ovaries on that plant or as a result of the lodging of wind-borne spores between the palea or lemma and the developing seed within.

It seems desirable in this connection to record here a simple preliminary experiment very strongly indicating that at least this new race of *Ustilago striaeformis* on *Agropyron* and *Elymus* in the Northwest is seed borne. As

already explained several plants of slender wheatgrass, infected with stripe smut (original collection L-A), were transplanted from the field to the greenhouse where they continued to produce smutted leaves and culms. On each plant were a few culms, on which at least some seed was produced. Some of these seeds were harvested so as to obtain a smut-free stand of a susceptible host on which stripe smut could be propagated, it being remembered that Davis (11) had shown that, at least as far as the smut on timothy is concerned, the organism is not seed-borne. These seeds were sown in the nursery in the spring of 1937.

The writer was surprised to find that *every plant in the nursery row in which the seed had been sown was infected with stripe smut*, and not one survived to produce any seed.

This experience and the fact that, as has been shown above, seed inoculations have been so successful, indicates that at least this race of *Ustilago striaeformis* is certainly seed-borne. This aspect of the life history should be investigated with regard to other races of *U. striaeformis*.

DISCUSSION

A comparison of the results here reported on life-history and inoculation studies of *Ustilago striaeformis* with those of earlier investigators makes it obvious that the collections L-A and L-B, from *Agropyron pauciflorum* and *Elymus glaucus*, respectively, constitute a race of this smut fungus that is very different from any heretofore investigated. This race differs from other races that have been studied in its pathogenicity, process of spore germination, physiological requirements for germination, and its ability to grow saprophytically. Furthermore, aside from a very few fusions between atypical non-abstricted lateral sporidia observed by Davis (9), studies of this race provide the first information we have had regarding the sexuality of *U. striaeformis*.

The fact that the collections L-A and L-B of *Ustilago striaeformis* could not infect redtop, Kentucky bluegrass, orchard grass, and timothy proves that those collections are pathogenically distinct from any of the races described on these hosts. Inasmuch as no inoculation experiments have been heretofore reported dealing with collections of *U. striaeformis* on *Agropyron* and *Elymus* spp., it seems probable that collections L-A and L-B represent a new race of this smut species, with a comparatively wide host range of grasses in the genera *Agropyron*, *Elymus*, *Hordeum*, *Sitanion*, and perhaps others.

The production and abstriction of typical sporidia, which characterizes this race, has not been heretofore described for *Ustilago striaeformis*. The development of 2 or 3 or more germ tubes or promycelia from the same spore is also new to *U. striaeformis*. According to earlier descriptions of germination in this species, a single elongate promycelium is extruded through a crack in the spore wall, which bears lateral branches or "primary sporidia" that are not detached.

The after-ripening period of several months in a moisture-saturated atmosphere at room temperature, which Davis (9) has shown to be prerequisite to successful spore germination in the races of *Ustilago striaeformis* that he studied, apparently does not apply to this new race on *Agropyron* and *Elymus* spp. in the Northwest. In the studies here reported, successful germination of fresh spores was nearly always possible, or if fresh spores failed to germinate well, several weeks' dry storage at room temperature usually sufficed to induce good germination. However, the fact that in a few cases fresh spores of this new race of *U. striaeformis* failed to show more than a trace of germination, and the fact that in one instance a collection of this smut from Kentucky bluegrass showed 85 per cent germination after 2½ months' dry storage and without any after-ripening period under moist conditions, indicates that we do not yet have a complete understanding of the physiological requirements for germination of the spores of this smut fungus.

This new race of *Ustilago striaeformis* is easily cultured and maintained on artificial media, which is in marked contrast to all other races studied heretofore in which the investigators could not induce the slightest saprophytic development. In the present studies vigorous, rapidly-growing cultures were easily obtained.

The data concerning the sexuality of the sporidia presented in this paper establish our first knowledge of the rôle of sex in the life history of *Ustilago striaeformis*, wherein it is seen that this smut fungus, although, in the symptoms produced, differing widely from most smut fungi that have been so investigated, is, nevertheless, very similar to these with respect to sporidial fusions, development of infection hyphae, and the initiation of infection by these hyphae. In other words, it appears that the fundamentals of the life history of *U. striaeformis* are not significantly different from those of other species of *Ustilago*.

Considering the extent to which the race of *Ustilago striaeformis* on *Agropyron* and *Elymus* in the Northwest differs from those studied elsewhere and on other grasses, it might be considered that these races do not all belong to the same species; indeed, there might be some justification in treating this new race in the Northwest as a separate, probably new, species. However, the fact remains that the morphology of the spores of this race is not significantly different from that of the spores of races on other hosts. It is the opinion of the writer that, for this reason, and because they all produce the same symptoms on the host plants, the race of *U. striaeformis* described in this paper should not be considered a valid species distinct from the type of *U. striaeformis*.

Since the collections L-A and L-B, from *Agropyron pauciflorum* and *Elymus glaucus*, respectively, represent a new race of *Ustilago striaeformis* it seems desirable to give it some designation. Davis (13) distinguished 4 physiologic races of *U. striaeformis*: (1) forma *Phlei*, on *Phleum pratense*; (2) forma *Agrostidis*, on *Agrostis palustris*; (3) forma *Poae-pratensis*, on *Poa pratensis*; and (4) forma *Poae-annuae*, on *Poa annua*. Following Davis'

classification (12), and considering that this new race parasitizes several genera of the tribe Hordeae, it should perhaps be designated as *Ustilago striaeformis* forma *Hordei*.

Although experimental evidence is somewhat lacking, it seems probable that stripe smut is seed-borne. This supposition is supported by the fact that seed harvested from non-smutted spikes, borne on an infected plant of *Agropyron pauciflorum*, yielded 100 per cent smutted plants. Inasmuch as the spores are developed and liberated throughout the period of blossoming and seed development, it seems probable that during the development of the seed the stripe-smut spores become lodged beneath the palea and lemma and either remain dormant there until the seed germinates, or may germinate immediately and develop in the pericarp or hull of the seed a mycelium, which becomes dormant as the seed approaches maturity. Thus, this phase of the life history of *Ustilago striaeformis* probably is much the same as in some of the cereal smuts, such as *U. avenae* and *U. nigra*. Therefore, stripe smut in slender wheatgrass, and in other species of *Agropyron* and *Elymus*, as caused by the race *Hordei* of *U. striaeformis*, should be subject to control by the usual fungicides recommended for control of certain seed-borne smut fungi of cereals. Experiments are in progress to determine seed treatment methods of control of stripe smut under Northwest conditions.

SUMMARY

This paper deals chiefly with certain aspects of the life history, physiology, and pathogenicity of a new race of *Ustilago striaeformis* occurring on grasses of the genera *Agropyron* and *Elymus* in the Pacific Northwest.

No after-ripening period under moist conditions was found prerequisite to successful germination of the spores, such as has been previously found necessary (10, 11) in certain races of *Ustilago striaeformis*. The period of germinability extended over a period of at least 7 months.

The process of spore germination is described and illustrated. From the germinating spore 2 or 3 thick germ tubes emerge, rapidly elongate, and develop cross walls and branches. Typical elliptical sporidia are budded from this complex promycelium in abundance. These sporidia possess the ability to develop rapidly into large colonies on agar media. Any saprophytic existence has been heretofore unknown in *Ustilago striaeformis*.

The sporidia are unisexual, representing one or the other of two sex groups. When sporidia of opposite sex are mixed together, especially on non-nutrient agar, they fuse within a few hours. From each fused pair there arises a long, vigorous, aerial infection hypha.

The sporidia of this race of *Ustilago striaeformis* fuse readily with certain other species of *Ustilago*. Mating experiments with 12 pedigreed monosporidial cultures of *U. bullata* and 4 of *U. striaeformis* showed: (1) The sporidia of *U. striaeformis* are highly compatible with those of *U. bullata*; (2) certain combinations are far more productive of infection hyphae than are others; and (3) in every case this greater number of infection hyphae involved the same sex group of *U. striaeformis*.

Ustilago striaeformis has been artificially cultured for the first time. Four monosporidial cultures (2 of each sex group) have been easily maintained on a variety of agar media. These cultures have continued a vigorous saprophytic development for over a year, having been transferred several times during this period. Although excellent growth is easily obtained on a variety of agar media, the optimum development has resulted on a 2 per cent agar containing 8 per cent dextrose, 4 per cent malt extract and 1 per cent peptone.

Inoculation experiments have been easily conducted, using aqueous suspensions of either spores or of sporidia of opposite sex as inoculum, by the partial vacuum method. High percentages of infection resulted on several species of *Agropyron*, *Elymus*, *Hordeum* and *Sitanion*. *Agropyron smithii*, *A. subsecundum*, *Elymus canadensis* var. *robustus*, *E. sibiricus*, *Hordeum jubatum*, and *H. nodosum* are reported as new hosts to *Ustilago striaeformis*, on the basis of inoculation experiments.

Inoculation experiments were completed in the greenhouse almost as successfully as in the field. In either case smut began to appear within 6 weeks after seeding.

Apparently, this race of *Ustilago striaeformis* is seed-borne, which has not previously been demonstrated for this species. Seeds taken from infected plants of slender wheatgrass produced only smutted plants.

Thirty-four selections and collections of slender wheatgrass were tested for resistance to this race of *Ustilago striaeformis*, with the result that most proved to be quite susceptible. Only 6 selections or collections appeared promising as resistant or immune stock.

The need for some designation of this new race is recognized, and it is recommended that it be known as race *Hordei*, or *Ustilago striaeformis* forma *Hordei*, in keeping with the classification of physiologic races of this species that Davis (12) has already initiated. In spite of the striking differences from other races of *U. striaeformis*, with regard to (1) spore germination and factors affecting the process, (2) pathogenicity, (3) culturability, (4) sexuality, and (5) seed carriage of the organism, this new race is indistinguishable, on the basis of spore morphology, from other races, and probably should not be considered a distinct species.

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CORTICIUM AREOLATUM, THE CAUSE OF THE AREOLATE LEAF SPOT OF CITRUS

GEROLD STAHEL

(Accepted for publication August 17, 1939)

Areolate leaf spot of *Citrus* is common in Surinam. Already, 20 years ago, the sour-orange stock of the nurseries was badly affected by this disease during long and heavy rainy seasons.

In 1929 Bondar (2) in Bahia described this leaf spot for the first time as "mancha areolada."¹ In his opinion it is the same leaf spot that occurs in Italy, and, according to Penzig, is caused by *Leptosphaeria citricola*.

Bitancourt and Jenkins (1) studied this disease again in 1935 and supposed the cause might be *Leptosphaeria bondari*. Infection experiments, however, were unsuccessful.

This *Leptosphaeria*, in Surinam, is very common on Citrus leaf spots. The same or a similar *Leptosphaeria* is found here as a common saprophyte on nearly every *Cercospora* leaf spot of the banana. It can be shown that the Citrus *Leptosphaeria* also is a saprophytic fungus.

The mycelium of the *Leptosphaeria* leaf-spot fungus grows rapidly and is, therefore, most easily isolated from young areolate leaf spots. This mycelium is entirely different from that of *L. bondari*. It resembles strikingly that of some primitive Basidiomycetes. Clamp connections are lacking.

To collect further and specific information about this parasite, I studied the epidermis of many of the very youngest leaf spots not yet necrotic. The germ tube was found to pierce the cuticle. Only once the spore was still attached to the germ tube (Fig. 4, H). It was a unicellular, thin-walled spore of basidiomycetous type.

It, therefore, seemed very probable, that areolate leaf spot of *Citrus* is caused by a representative of the Basidiomycetes.

OCCURRENCE AND SYMPTOMS

Not all varieties of *Citrus* show the same susceptibility to attack by the areolate-leaf-spot fungus. Sour orange used for stock, suffers most severely. Grapefruit, pomelo, mandarin, and king also are susceptible. The common orange tree is fairly resistant, though, under heavy shade of *Erythrina glauca*, even with oranges, many leaves may be spotted during a long rainy season.² On lemon, lime, succade, and kumquat, I never observed areolate leaf spot.

Only the very young, immature leaves are subject to infection during rainy weather. In Surinam, new shoots and twigs may appear on citrus

¹ In his original publication (p. 74) Bondar corrected "areolada" with ink into "aureolada." The term "areolate leafspot" thus originated from a misprint, but is used now exclusively. It seems to me better to perpetuate this and not to confuse the literature with the rehabilitation of the original name.

² In Surinam, full-grown orange trees, if not irrigated, suffer more or less from dieback. Under light shade of *Erythrina glauca*, the shade tree used here for coffee and cacao, dieback of Citrus may entirely be prevented, even without irrigation.

trees throughout the year, excepting the dry season. If the new twigs grow out during a fortnight with none or only a moderate rainfall, all the leaves remain permanently healthy. During continuously rainy weather, however, the new leaves become successively more and more spotted, and, ultimately, so much so that all are shed. By that time a grapefruit tree shows plenty of old, dark-green, healthy leaves, only the new ones being spotted or shed. The full-grown trees of the susceptible varieties, therefore, suffer severely only in years of abnormally long, rainy seasons. In the nurseries of sour-orange stock, however, this disease causes much more trouble; in wet years, the plants may be badly defoliated if no control measures are taken.

The primary dead spots of $\frac{1}{2}$ –1 mm. diameter appear, just as the leaf becomes full-grown, but still shows the light-green color of the immature leaf. In this stage the spots may begin to enlarge and continue to do so for many weeks during rainy weather.

The very first symptoms are small light-green spots on the young, not yet full-grown and still soft leaves. These spots die after some days and form the above-mentioned necrotic primary spots (Fig. 1, A). In heavily infected nurseries I counted 100 and more of these primary spots on a single leaf. If this stage is reached during days of heavy rain, most of the spots enlarge and the leaf is shed after some weeks. If, however, the weather is dry, all the spots remain permanently at this stage, causing no trouble of any importance.

The enlarging spots add every day one new ring to the spot. During 2 weeks I followed daily the growth of 12 spots. Every morning one new water-soaked ring about 1 mm. broad was found around the spot. During the day this ring discolours into brown and the tissue collapses. At the same time the leaf cells contiguous to the spot are impregnated by a yellowish gum. This gummed barrier is not yet tight in the evening of the first day, so that during the night, if sufficient moisture is present, the barrier is passed by the fungus and a new water-soaked ring is added. The rings of gummed cells do not collapse and form the concentric ridges so typical for areolate leaf spot. These ridges are more prominent on the lower than on the upper side of the leaf.

During dry weather the fungus stops growth and the gummed barrier is closed. Further spreading of the fungus inside the leaf is impossible. Sometimes, however, if wet weather prevails, the fungus may still find one or a few breaches to pass through. In this case it forms the curiously branched spots, that may be found sometimes. Usually, the spots show 10–20 rings, but in an extreme case I counted as many as 47 rings on a branched spot.

The enlarging of the primary spots seems to be more difficult than that of the ring spots. On most of the older spotted leaves several of these inconspicuous primary spots may be found. But even if they expand, they usually do so asymmetrically. Purely concentric spots are rare.

The color of the spots is light-brown; that of the ridges, dark-brown.

The upper surface is glossy, the lower dull. Around the spots the leaf is discolored, becoming yellowish.

During wet weather the lower side of many of the spotted leaves is covered with a whitish mildew (Fig. 1, B). There may be only a few patches outside the dead spots, sometimes, however, a big part, exceptionally the whole leaf, may be covered with it. Even the dead spots may show the mildew. These patches are nearly always very inconspicuous, especially on the light-

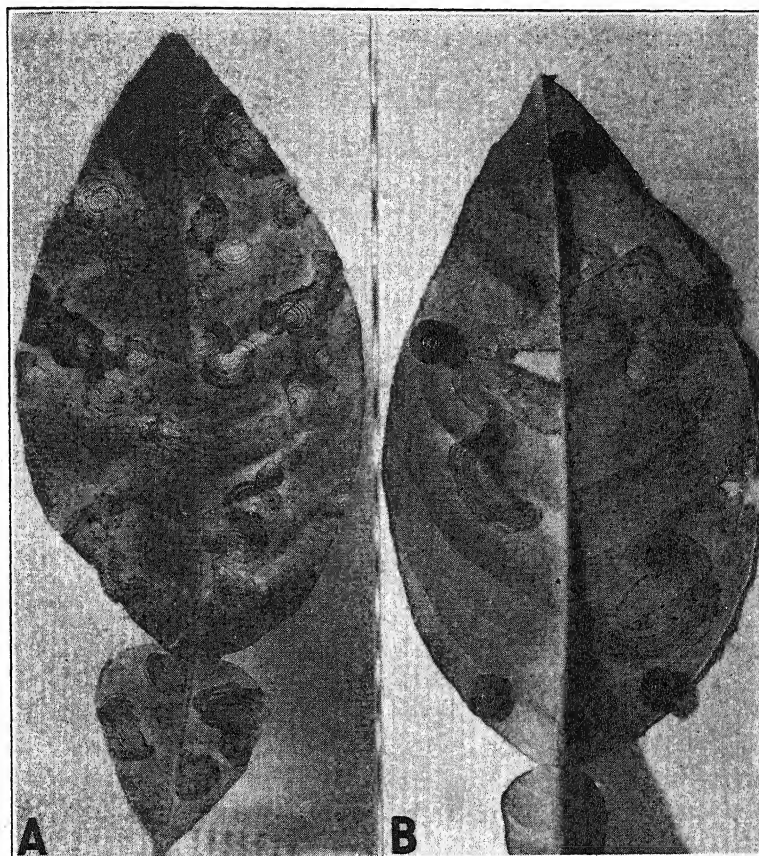


FIG. 1. A. Sour orange leaf with areolate leaf spots and 22 primary, not developed spots. B. Lower surface of sour orange leaf. On the right in the middle note the epiphylllic basidiophorous mycelium.

green, dull, lower leaf surface. It was Bitancourt who, during my visit to São Paulo, in October, 1938, called my attention for the first time to this very important symptom of areolate leaf spot. During very wet weather it sometimes happens, that even the upper surface of the leaf may show big patches of mildew, easily visible on this dark-green side of the leaf.

During wet weather the mycelium in the dead spots of the fallen leaves grows profusely out into the soil, and it is not without some effort that such a leaf can be detached from the underlying earth. Inspecting the lower

side of such a leaf, the spots appear to be covered with sand, and strands of hyphae covered with particles of sand hang down from the spots. As with other *Corticiums*, the areolate leaf-spot fungus is apparently a soil fungus. It is very probable, indeed, that it survives the long dry season in the soil in the form of sclerotia to reinfect the Citrus leaves at the commencement of the rainy season.

On young, green twigs primary spots may be found, as mentioned for the first time by Bondar (2). They never enlarge.

On the fruits I never detected even primary spots, though it is not impossible, that they may be found there.

The oldest parts of the spots are generally covered by small black points, the perithecia of *Leptosphaeria bondari*, a common saprophyte.

THE CAUSAL FUNGUS

The fungus is easily isolated. A piece of a young areolate leaf spot, about $1 \times \frac{1}{2}$ cm., is transferred to slant agar (Sabouraud). Within an hour the fungus grows all around into the agar. After 15 hours a strong mycelium, 1 cm. wide, surrounds the piece. From the edges the pure mycelium may be transferred to new agar tubes. If a young, clean leaf spot be selected, no superficial disinfection is needed. I used only a disinfected knife and a sterile Petri dish to cut the piece.

After about 4 days the mycelium covers the surface of the slant agar in the test tube. It is first hyalin but discolors quickly into a greyish brown. A low, rough, aerial mycelium covers somewhat irregularly the surface of the agar. The mycelium grows over the free surface of the glass, where it forms a fine, transparent, closely adhering mycelium of one layer of hyphae. On this mycelium some strains produce plenty of sclerotia of about 1 mm. diameter; commonly, however, no sclerotia are formed in agar tubes. They are found most abundantly, when cultivated on sterilised potato slices (Fig. 2); but, even here, some cultures show only a few or no sclerotia at all.

Young sclerotia are whitish or light brown; later, they discolor into dark brown.

The sclerotia are formed by a clump of oidium-like, much branched, and curled hyphae, being lateral branches of the straight hyphae that creep over the glass. Its structure is pseudoparenchymatic. Not the slightest trace of a cortex is present. Even in the centre, the different oidium-like hyphae are easily distinguished, though the intercellulars are not wide. In the outer parts of the sclerotium some of the common hyphae may be found from which the swollen sclerotial hyphae arise.

I never found these sclerotia outside the pure cultures, but I strongly suspect that they are formed in the soil by the mycelium that grows out so abundantly from the spots of the fallen leaves.

On the potato slices a white, feathery, aerial mycelium appears consisting of strangely branched hyphae.

In the leaf spots the fungus fills up practically all the spaces between

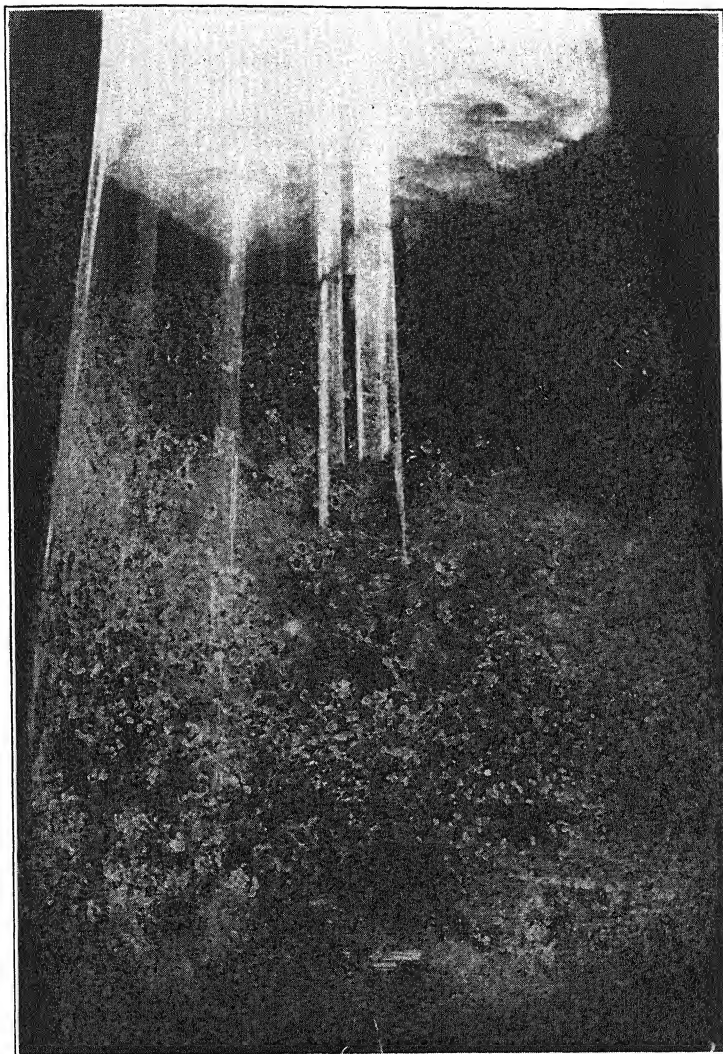


FIG. 2. Sclerotia on the inner surface of a wide mouth Erlenmeyer flask. Culture on potato.

the mesophyll cells. Short side branches penetrate between the palisade cells. The hyphae here grow tortuously, forming knots.

During rainy weather this mycelium grows out from the spots and spreads over the surface of the living parts of the leaf, as mentioned above. It is the same cobweb-like mycelium, that grows over the glass surface in pure cultures. Here however, the oidium-like side branches do not clump to form sclerotia but spread on the surface, producing on the lateral branches many isolated, short basidia (Figs. 3 and 4 D). During continuous heavy rainy weather the basidia produce 4 sterigmata, 10-13 μ long, with a 3-3½ μ

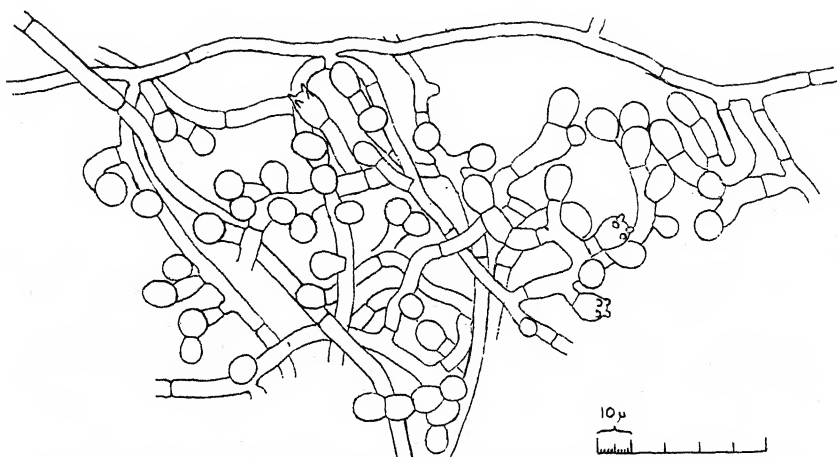


FIG. 3. Typical basidiophorous mycelium from the lower surface of the leaf.

broad base (Fig. 4, B and C). The spores are hyalin, smooth, $5 \times 8-9 \mu$, with a papilla (Fig. 4, E and F). There are regularly 4 sterigmata, only

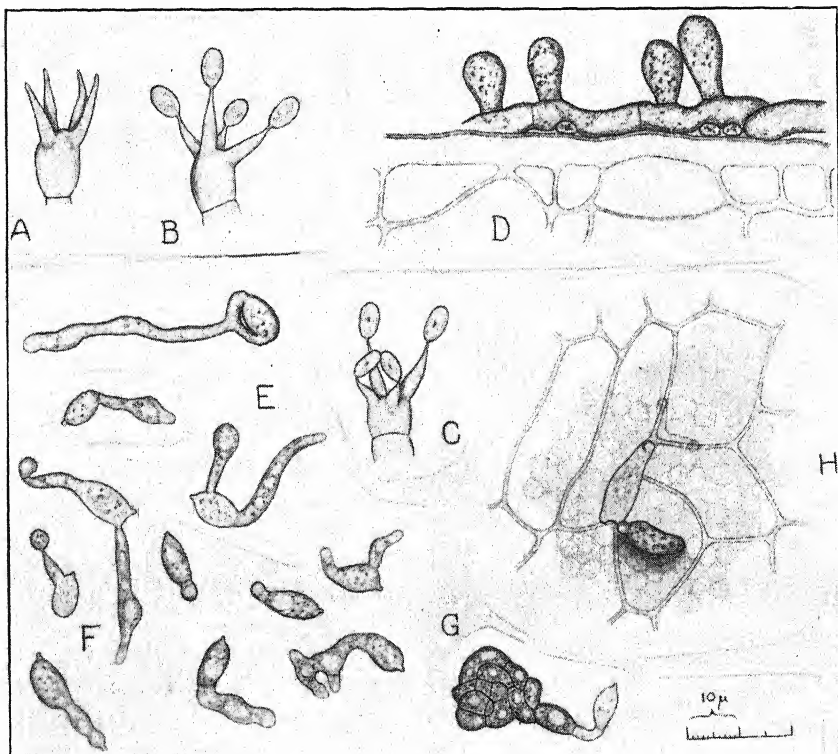


FIG. 4. A-C. Sporulating basidia. D. Epiphyllie mycelium with basidia. E. Ten basidiospores germinated on Sabouraud's agar. F. Basidiospores germinated in water. G. Basidiospore germinated on Sabouraud's agar forming a sclerotium, fourth day after germination. H. Germinated basidiospore on sour orange leaf showing a small appressorium, a flat subcuticular hypha, and hyphae in the palisade layer.

once I observed a basidium with 2. The basidia sporulate in groups, wherein every fifth to tenth may show sterigmata.

Bondar found this mildew commonly associated with areolate leaf-spot, and named it *Oidium citri*. Bitancourt confirmed this and supposed already in 1933 (in a letter to H. S. Fawcett), that this *Oidium* may be a *Corticium*

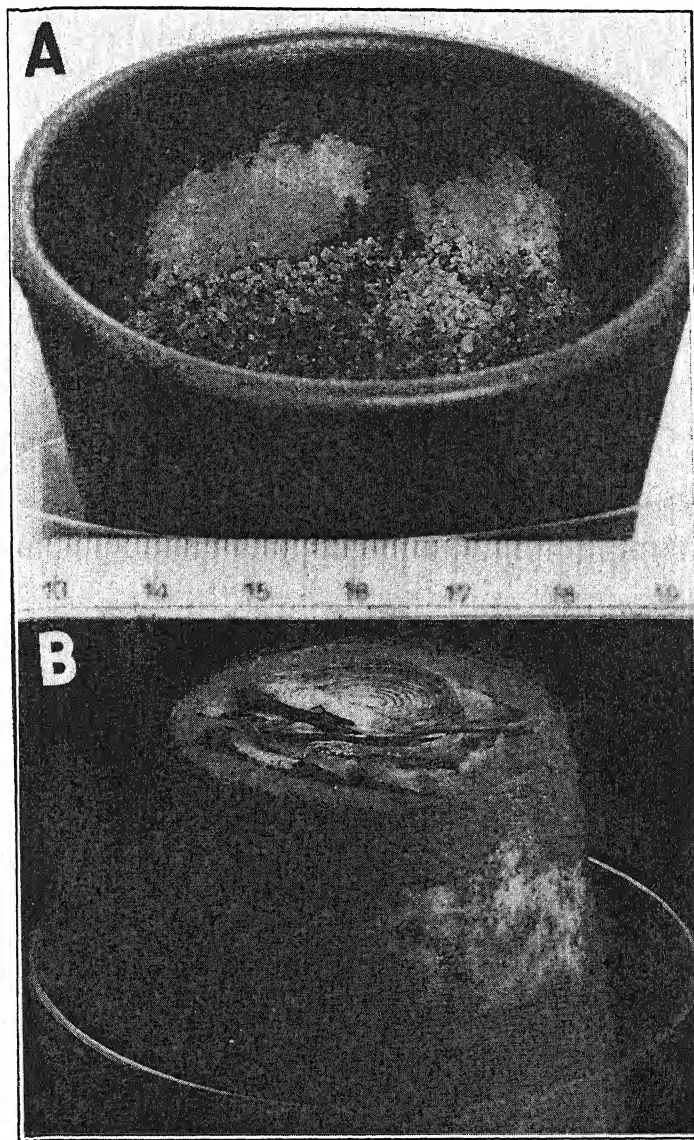


FIG. 5. A. Small flowerpot filled with moist sand. The buried sclerotia are grown out to form basidiophorous mycelia on the inner surface of the pot as well as on the sand. B. Moist flowerpot with basidiophorous mycelia grown out from about 12 areolate leaf-spots on the top of the inverted pot.

belonging as chief fructification to the leaf-spot fungus. The fact, however, that he couldn't find sporulating basidia nor clamp connections was the reason that he didn't mention this supposition in his publication written together with A. E. Jenkins in 1935 (1).

I collected the spores in Petri dishes on microslides. Inside the cover several pieces of leaves, covered with mildew, were fixed by droplets of water. If too many pieces are present in the same dish, the air becomes saturated with water and sporulation stops. In this case the epiphylllic hyphae grow out into a cotton-like aerial mycelium. Under adequate conditions the production of spores is most prolific, if the leaves are collected during continuous rainy weather. Then, within 24 hours, the microslides become covered with a whitish spore powder, easily visible to the naked eye.

Before I discovered the basidia on the leaf surface, I cultivated them from sclerotia produced on thick slices of potato in Petri dishes. The sclerotia on the inside of the cover were scraped together with a scalpel, brought into small flowerpots half filled with clean sterile sand, and buried 1-2 cm. deep. The whole was left moderately wet and uncovered, so that fresh air could circulate freely.

After 10-14 days the inside surface of the flowerpot showed big patches of a white mildew exactly like that on the leaves (Fig. 5, A). Sporulation, however, is rather more prolific on the flowerpots than on the leaves, apparently because the moisture is here more constant and may be regulated more conveniently. The basidia also are produced on the surface of the sand.

In the same manner the mycelium in the soil of a nursery or an orchard may grow out at the commencement of the rainy season and produce basidia on the surface. These basidiospores reinfect the nursery plants. A heavy outbreak of the disease, however, is not possible before plenty of leaves are covered with mildew produced by the dead spots.

It is not necessary to bury the sclerotia in the sand. The same thing happens if the sclerotia are put on a continuously and moderately moist inverted empty flowerpot. Instead of sclerotia I used with the same success young areolate spots cut out from spotted leaves (Fig. 5, B). The sclerotia and leaf spots, however, have to be covered during the first week with a small glass plate to retain the moisture.

These experiments show the following conditions necessary to the production of the basidiophorous mycelium: *a.* A well-nourished mycelium. *b.* A moderately but constantly moist substratum. *c.* An unhindered ventilation in the open air.

Here the temperature is not considered. For Surinam, with about the same temperature throughout the year, this factor is of no importance. The fact, however, that, according to Bitancourt, the sour orange in Santos suffers from this disease, whereas in the cooler climate of nearby São Paulo no areolate spot at all is known, shows that the minimum temperature may be fairly high. The areolate leaf-spot fungus needs a wet, tropical climate.

INFECTION EXPERIMENTS

In a citrus nursery, even if badly infected with areolate leaf spot, all the young and tender leaves are free from spots. But when the leaf has just reached its normal size, the primary spots appear. On old leaves young growing spots never are found. It is clear, therefore, that only the young, tender leaves are subject to infection by basidiospores.

For the infection experiments, I used mycelia, sclerotia, and basidiospores. With all infection was successful.

To inoculate sclerotia and mycelia, I made 2-3 cm. cuts in old dark-green leaves, using a razor to make the cuts. Immediately small quantities of sclerotia or mycelia, cleaned carefully from adhering agar, were brought into the cuts. Two days later the edges appeared to be necrotic and later, every day, a new ring was added, producing the typical areolate leaf spots (Fig. 6). Inoculations with mycelium grown on nutrient agar from basidiospores had the same result.

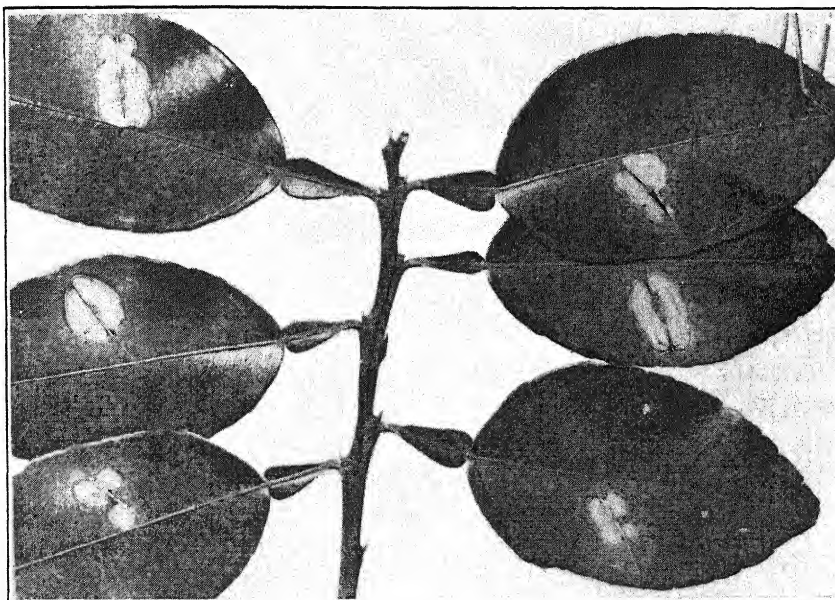


FIG. 6. Infection of old leaves with sclerotia brought into cuts freshly made with a razor.

To infect the young leaf with basidiospores I suspended the small flowerpots and spotted leaves covered with sporulating basidia above a developing new shoot of a young grapefruit plant. The whole was covered with a big flowerpot kept moist constantly. With the spores of both origins infection was successful. The young grapefruit plants were growing close to my laboratory and were free from leafspots for at least 10 months. Infection was also successful when the spores, shed from the epiphyllie mycelium in Petri dishes, were collected with a wet pencil and brought on the leaves.

These inoculations, however, were commonly less effective, than those with spores directly shed on the leaves.

The experiments have shown, that only the youngest leaves of less than half the full-grown size take infection. Both sides of the leaf are susceptible. The incubation time is about 14 days.

In water and nutrient agar the basidiospores germinate easily within 12 hours. The germ tubes grow very slowly, forming—especially on nutrient agar—irregular swellings and windings, typical for the germ tubes of many parasitic fungi.

On nutrient agar after 4–5 days the germ tube forms a small sclerotium (Fig. 4, G). From this sclerotium the hyphae grow out and spread over the agar, producing the mycelium.

About the same happens if the fungus enters the leaf. The basidiospore germinates immediately, producing a minute appressorium that pierces the cuticle (Fig. 4, H). The resultant germ tube then grows as a flat hypha for a short distance between the cuticle and the epidermal cells and then

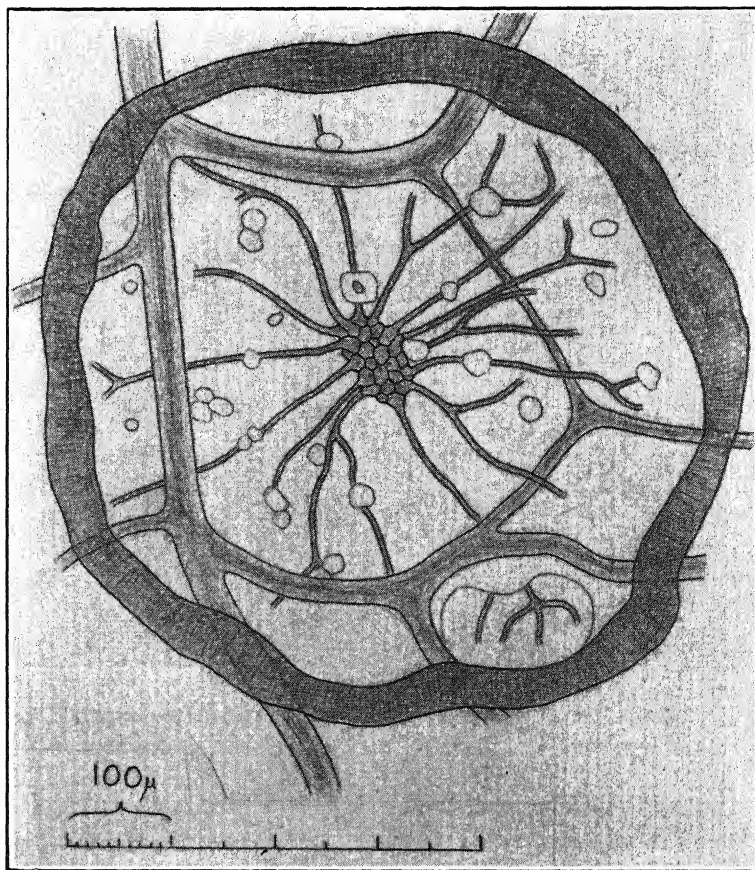


FIG. 7. Primary spot with the small initial sclerotium and the spreading hyphae. Gummed ringwall, nerves, and crystal cells.

enters the deeper leaf tissue by way of the intracellular space between epidermal cells.

In the mesophyll just below the point of spore germination, a sclerotium is formed that kills the tissue when the leaf is just full-grown. Then all round the edges of the sclerotium the hyphae spread radially into the dead tissue, and, if the weather is wet enough, this primary spot grows out to an areolate leaf spot as described above (Fig. 7).

The fungus doesn't form a true hymenium. The basidia are scattered over the side branches of the fertile mycelium (Fig. 3) that covers the substratum like a cobweb.

Accepting the reclassification of the *Thelephoraceae* by Burt (1914) the fungus is a *Corticium* and not a *Hypochnus* (with echinulate spores). I, therefore, propose to name the fungus causing areolate leaf-spot of Citrus *Corticium areolatum*, nov. spec.

Description of the Fungus

Vegetative hyphae brown, 5-8 μ , maximal 10 μ thick, distance of septa 50-200 μ , exceptionally 320 μ , clamp connections lacking. Hyphae in the leaf spot tortuous and knotty, epiphyllic hyphae straight, light brown, with hyaline ramified side branches of swollen cells bearing basidia. True hymenium and pileus lacking. Basidia short 10-14 \times 8-10 μ , sterigmata 4, exceptionally 2, 10-13 μ long, basis of the sterigmata 3-3½ μ thick, spores smooth, hyaline, papillate 5 \times 8-9 μ .

Sclerotia whitish or light-brown, later dark brown; diameter 1 mm., generally somewhat flat, consisting of a clump of swollen hyphae, cortex lacking. Observed only in pure cultures on the surface of the glass container.

Wet-weather parasite on citrus leaves, especially on sour oranges, but also on grapefruit, pomelo, king, mandarin. Fairly immune are oranges, immune lemon, lime, succade, kumquat.

In Surinam in the coastbelt, causing brown leaf spots, showing typical concentric rings, called "ringvlekkenziekte." Known as "mancha areolada" (mancha aureolada) and "areolate leaf spot," in Brazil from Bahia to Paraná and also in Venezuela (H. S. Fawcett, Citrus diseases, 1936).

Hyphis vegetis brunneis, 5-8 (-10) μ crassis, in maculis tortuosis nodosisque, epiphyllis rectis, pallide brunneis; ramis lateralibus hyalinis, ramosis, e cellulis inflatis basidiiferis compositis; hymenio et pileo deficientibus; basidiis curtis, 10-14 \times 8-10 μ ; sterigmatibus 4, rare 2, 10-13 μ longis, ad basim 3-3.5 μ crassis; sporis levibus, hyalinis, papillatis, 5 \times 8-9 μ ; sclerotiis in culturis pallidis dein brunneis, 1 mm. diam., fere leniter deplanatis. Maculas in foliis Citri producents, Surinam.

CONTROL

In nurseries of sour orange stock this very troublesome disease may effectively and economically be suppressed by collecting and burning all the spotted leaves. In Surinam this work is done by women and children.

If a nursery has to be sprayed for scab (*Elsinoë fawcetti*), areolate leaf spot is controlled too. Bordeaux mixture kills and impedes the development of the sporulating epiphyllic mycelium.

During a long continuous rainy season grapefruit orchards suffer badly from this disease. A thorough spraying of the soil under the trees in the beginning of the rainy season may prevent or retard reinfection from the soil. If, in spite of this treatment, the new leaves are heavily spotted before the end of the rainy season, the spotted leaves have to be sprayed during dry days, especially on the lower side.

AGRICULTURAL EXPERIMENT STATION
PARAMARIBO, SURINAM

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PRELIMINARY SEROLOGICAL STUDIES OF PHYMATOTRICHUM OMNIVORUM¹

R. W. CUMLEY AND G. W. GOLDSMITH

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Although the cotton root-rot fungus has been the subject of numerous reports appearing during the past 30 years, the life history, including the perfect stage, has not been established. Both Shear (4) and Duggar (1) placed the fungus in the Hyphomycetes. Later, Shear (5) reported hyphal connection between diseased cotton and osage orange, upon which sporophores occurred, and assigned to the fungus the name *Hydnum omnivorum*. This result has not been confirmed. Taubenhaus and Ezekiel (6) reported negative results. Recently, Presley and Thom (3) have made the suggestion that the fungus is to be regarded as a Gasteromycete, and the spore mats, previously considered conidial, are in reality the puffball sporophores. It is obvious that the correct classification of the fungus and interpretation of the structures would be of considerable theoretical and practical importance.

A preliminary serological study was undertaken to determine the relationship of *Phymatotrichum omnivorum* (Shear) Duggar to the various members of different groups of fungi. Two methods of securing material were followed. In one the young growing sporophores were collected in the field and immediately washed and dried over sulphuric acid at 50° C., and stored in sterile glass jars at 5° C. The following were prepared in this way: *Tyromyces palustris* (Berk. & Curt.) Murrill, *Psalliota silvatica* (Schaeff.) Quél, *Clitocybe illudens* Schw., *Lycoperdon gemmatum* Batsch, *Calvatia cyathiformis* (Bosc.) Morgen, *Secotium acuminatum* (Mont.), *Ustilago maydis* (DC.) Cda.

In the second method pure cultures of fungi were grown in liquid culture, removed when the growth was well developed over the surface, washed, and dried under the conditions just mentioned. By this method *Rhizopus nigricans* Ehrenberg, *Aspergillus niger* group Thom and Church, *Penicillium luteum* group Thom, *Hormodendron cladosporioides* (Fres.) Sacc., *Fusarium* sp., *elegans* section Gilman and Abbott, *Sclerotium rolfsii* Sacc., and *Phymatotrichum omnivorum* (Shear) Duggar were obtained.

MATERIALS AND METHODS

Culture Medium for Phymatotrichum omnivorum.—The fungus mats

¹ Contribution from The Clayton Foundation.

were grown on an artificial nonprotein medium composed of the following materials dissolved in the order given:

Distilled water, 1000 cc.; dextrose, 40.00 g.; ammonium nitrate, 1.8 g.; di-potassium phosphate, 1.35 g.; magnesium sulphate, 0.75 g.; potassium chloride, 0.15 g.; and iron chloride (0.5 per cent solution), 0.25 cc.

Cultures were grown at room temperature in 2-liter Erlenmeyer flasks containing about 400 cc. of culture media. The fungus mats were collected at approximately 1 month after seeding.

Collection of Fungus Mats.—The mats were recovered by decanting the culture medium and pouring the mat into a large bowl of tap water. After 2 or 3 rinsings in tap water, the mat was rinsed twice in distilled water and dried rapidly in an incubator at 40–45° C. About 6 hours, and never more than 12, were required to get the material to constant weight. The mats were then placed in air-tight jars and stored at 5° C.

Preparation of Injection Materials.—Injection antigens were prepared in a manner similar to the technique of Link and Wilcox (2) working with species of *Fusarium*, *Sclerotinia*, and others. The technique employed was as follows: Physiological saline (.85 per cent NaCl) was added to the dried and powdered fungus material in the proportion of approximately 33 parts saline to 1 of fungus. The mixture was shaken thoroughly and placed in the ice-box at 5° C. for about 18 hours. During the course of the extraction the material was shaken several times. After extraction, the mixture was centrifuged and the clear amber-color supernatant liquid collected by decantation. To this extract was added merthiolate solution (1:1000) in the proportion of 9 parts of extract to 1 of merthiolate. The resulting fluid constituted the intravenous injection material and was preserved in the ice-box. This solution contained 0.0135 gm. fungus powder per cc.

The material for intraperitoneal injection was prepared from the residue remaining when the aforementioned extract was collected, after centrifugation. This residue was dried and weighed. It was then ground extremely fine in a mortar and resuspended in saline in the same proportions as in the preceding extraction, addition of merthiolate and preservation at 5° C. being the same as already described. Unlike the prior extraction, this mixture never assumed an amber color, but remained a dull gray caused by the suspended particles. This suspension contained 0.03 g. of preextracted fungus powder per cc.

Inoculation of Rabbits.—Three rabbits were inoculated 6 times, intravenously, with the above described solution and, intraperitoneally, with the suspension. The injections were given at about 4-day intervals. Table 1 is a presentation of the immunization schedule.

Seven days after the last injection the animals were starved for 24 hours and then bled from the heart. The amount of blood taken from the rabbits varied from 15 to 45 cc. The blood was allowed to remain in the ice-box overnight, after which the serum was pipetted into sterile containers and kept at 5° C., preserved with merthiolate solution. These sera were the anti-

TABLE 1.—Immunization schedule followed in inoculating 3 rabbits 6 times intravenously and intraperitoneally with injection antigens

Date	Rabbits						Remarks
	Number 1		Number 2		Number 3		
	Intravenous (solution)	Intraperitoneal (suspension)	Intravenous (solution)	Intraperitoneal (suspension)	Intravenous (solution)	Intraperitoneal (suspension)	
3-12-38	cc. 1.0	cc. 1.5	cc. 1.0	cc. 1.5	cc. 1.0	cc. 1.5	No. 1 had large knot on belly and was sick after shots. Small knots on belly of each rabbit. Knots on No. 1 smaller.
3-15-38	2.0	2.5	2.0	2.5	2.0	2.5	
3-18-38	3.0	2.0	3.0	2.5	3.0	3.0	
3-21-38	4.0	4.5	4.0	3.0	4.0	4.5	
3-25-38	4.0	3.0	4.0	4.0	4.0	2.5	All animals bled from heart.
3-28-38	5.0	5.0	5.0	5.0	5.0	4.0	
4-4-38							

Phymatotrichum sera that were employed in the subsequent tests with numerous fungus antigens.

Preparation of Test Antigens.—The antisera were tested with antigens prepared from 14 different genera of fungi, either collected in the field or grown on suitable synthetic media. Before extraction, the material was thoroughly dried, powdered, and weighed. The extracts used in the tests differed from the injection extracts in that the test antigens were prepared from dry powders, previously extracted with ether. The purpose of this extraction was to remove lipoids that have been thought to interfere with the specificity of the precipitin reaction.

The material prepared from each species was extracted 4 times with ether, after the technique of Link and Wilcox (2). This technique was as follows: "Ether was added to the desired amount of powder, shaken occasionally and allowed to act for 1–15 hours at 25° C. After centrifugation this ether was decanted and fresh added. Usually 3 or 4 changes sufficed to give a fat-free test when a drop of ether was evaporated on a watch crystal. The ether was then decanted and the powder thoroughly dried preliminary to saline extraction." Table 2 is a presentation of the various species employed here and an account of the percentage of ether-soluble material removed during the 4 ether extractions.

TABLE 2.—Data regarding ether-soluble constituents of several species of fungi

Name of fungus	Wt. of powder before ether- extraction	Wt. of powder after ether- extraction	Percentage ether- soluble constitu- ents removed by extrac- tion
	Grams	Grams	Per cent
<i>Aspergillus niger</i>	3.98	3.88	2.40
<i>Calvatia cyathiformis</i>	5.00	4.84	3.20
<i>Clitocybe illudens</i>	5.00	4.65	7.00
<i>Fusarium elegans</i>	0.67	0.38	43.30
<i>Hormodendron cladosporioides</i>	5.00	4.62	7.50
<i>Lycoperdon gemmatum</i>	5.00	4.82	3.50
<i>Penicillium luteum</i>	5.00	4.27	14.50
<i>Phymatotrichum omnivorum</i>	5.00	4.60	8.00
<i>Psalliota silvatica</i>	5.00	4.20	16.00
<i>Rhizopus nigricans</i>	2.00	1.75	12.50
<i>Sclerotium rolfsii</i>	0.23	0.21	8.60
<i>Secotium acuminatum</i>	5.00	4.70	6.00
<i>Tyromyces palustris</i>	5.00	4.02	19.50
<i>Ustilago maydis</i>	5.00	4.79	4.10

After the ether had been thoroughly removed from the fungus powder, 0.85 per cent saline was added in the proportion of 1 g. of powder to 40 cc. of saline. The mixtures were shaken at frequent intervals and remained in the refrigerator at 5° C. for 3 days. They were then centrifuged and the clear supernatant solution collected and filtered. The residue was quickly dried and weighed. Hence, the amount of material that had gone into solution during the course of extraction could be calculated. Table 3 presents the data regarding this saline-extraction of the pre-ether-extracted

TABLE 3.—Data regarding solubility in saline of fungal powders previously extracted with ether

Name of fungus	Powder pre-extracted with ether	Saline added	Extract removed	Residue remaining	Material in extract	Dilution of material in extract
	Gram	cc.	cc.	Gram	Gram	Gram/cc.
<i>Aspergillus niger</i>	1.000	40.0	33.0	0.815	0.185	1: 178.5
<i>Calvatia cyathiformis</i>	1.000	40.0	32.0	0.970	0.380	1: 97.0
<i>Clitocybe illudens</i>	1.000	40.0	36.0	0.327	0.673	1: 53.5
<i>Fusarium elegans</i>	0.375	14.4	10.0	0.272	0.103	1: 97.2
<i>Hormodendron cladosporioides</i>	1.000	40.0	35.0	0.835	0.165	1: 212.1
<i>Lycoperdon gemmatum</i>	1.000	40.0	33.0	0.592	0.408	1: 80.8
<i>Penicillium luteum</i>	1.000	40.0	33.0	0.576	0.424	1: 77.9
<i>Phymatotrichum omnivorum</i>	1.000	40.0	36.0	0.660	0.340	1: 105.8
<i>Psalliotia siliatica</i>	1.000	40.0	35.0	0.382	0.618	1: 56.6
<i>Rhizopus nigricans</i>	1.000	40.0	32.0	0.740	0.260	1: 123.1
<i>Sclerotium rolfsii</i>	0.210	8.4	7.2	0.157	0.053	1: 136.0
<i>Secotium acuminatum</i>	1.000	40.0	34.0	0.662	0.338	1: 100.5
<i>Tyromyces palustris</i>	1.000	40.0	31.0	0.440	0.560	1: 55.4
<i>Ustilago maydis</i>	1.000	40.0	34.0	0.832	0.168	1: 202.2

powders. After having computed the quantity of powders in the various extracts, the dilutions of all were adjusted, by the approximate additions of saline, to the ratio of 1:250. Merthiolate solution (1:1000) was then added to the solutions as a preservative. These extracts constituted the test antigens employed in the subsequent complement fixation and precipitin tests.

The Precipitin Test.—The ring precipitin test of Ascoli was applied to the antisera, in the attempt to differentiate the antigens prepared from the several fungus species. In this test the antiserum was carefully placed in the bottom of 2" × $\frac{1}{4}$ " precipitin tubes, to a depth of about $\frac{1}{8}$ ". The antiserum was held at the same dilution throughout the 12 tubes in the rack. The antigens were diluted serially, beginning with 1:250 (the stock solution) and proceeding up to 1:128,000. The antigen of the proper dilution was layered on top of the antiserum, care having been taken not to allow the 2 reagents to mix at the interface. Approximately the same quantities of antigen solution and antiserum were used in each tube. Readings were taken at frequent intervals up to 3 hours. The 2-hour reading appeared to be the most consistent and is used in the results reported in table 4. The faintest perceptible ring is indicated in the results as a +, whereas a heavy, thick, opaque ring is indicated as a +++. Negative results are indicated as —.

The Complement Fixation Test.—Before the complement fixation tests could be executed, the antigens were titrated for anticomplementary activity. This was accomplished by holding constant the amounts of complement (guinea pig serum), hemolytic antigen (2 per cent sheep cells), and hemolytic amboceptor (anti-sheep cell serum from rabbit), and serially diluting the antigen to be tested. The results of this test are shown in table 5. Nega-

TABLE 5.—Titration for anticomplementary activity of antigens

Species antigen	Antigen dilution						
	1: 250	1: 500	1: 1000	1: 2000	1: 4000	1: 8000	1: 16,000
<i>Aspergillus niger</i> ...	—	—	—	—	—	—	—
<i>Calvatia cyathifor-</i> <i>mis</i>	—	—	—	—	—	—	—
<i>Clitocybe illudens</i> ...	—	—	—	—	—	—	—
<i>Fusarium elegans</i> ...	—	—	—	—	—	—	—
<i>Hormodendron cla-</i> <i>dosporioides</i>	—	—	—	—	—	—	—
<i>Lycoperdon gem-</i> <i>matum</i>	++	++	—	—	—	—	—
<i>Penicillium luteum</i>	—	—	—	—	—	—	—
<i>Phymatotrichum</i> <i>omnivorum</i>	—	—	—	—	—	—	—
<i>Psalliota silvatica</i> ...	++++	++++	++++	++++	++	+	—
<i>Rhizopus nigricans</i>	—	—	—	—	—	—	—
<i>Sclerotium rolfsii</i> ...	—	—	—	—	—	—	—
<i>Secotium acumina-</i> <i>tum</i>	—	—	—	—	—	—	—
<i>Tyromyces palus-</i> <i>tris</i>	—	—	—	—	—	—	—
<i>Ustilago maydis</i>	++	—	—	—	—	—	—

tive—results indicate no interference with the action of complement, whereas ++++ indicates considerable and serious interference.

Because of the fact that the antigen prepared from *Psalliotia silvatica* was anticomplementary in a dilution of 1:8000, it was discarded and not tested with the subsequent complement fixation reactions. In order to avoid any possible anticomplementary action indicated by the *Ustilago maydis* and *Lycoperdon gemmatum* reactions, and at the same time to keep all of the antigens of the same strength, the solutions were all diluted to the ratio of 1:1500. With these antigens, the antisera were tested by the complement fixation technique. The antigen was held at constant dilution and the antiserum was serially diluted from 1:6 to 1:160. Preliminary tests with the homologous antigen indicated that the titre of complement fixation did not exceed that figure. In table 6, where the results of the several tests are compiled, ++++ indicates complete complement fixation, and — indicates no fixation of complement.

RESULTS

The Precipitin Test.—The precipitin test was employed with all 3 of the rabbit sera. The serum of rabbit No. 1 was cloudy and was found unsuitable for the test. The serum of rabbit No. 3 gave a precipitate with saline. Several titrations were made to determine the saline dilution that would not form a ring with the antiserum, without success. Rabbit No. 2, however, yielded a serum that was neither cloudy nor affected by saline. Furthermore, this serum precipitated the homologous antigen in high dilutions. The results reported in table 4 are compiled from many precipitin tests executed on this antiserum. In this table, one may observe that antigens prepared from *Lycoperdon gemmatum*, *Secotium acuminatum*, and *Calvatia cyathiformis*, in the several tests conducted on the rabbit No. 2 antiserum, always reacted more nearly as did the *Phymatotrichum* antigen than did any of the other antigens tested. Hence, one may conclude tentatively that *Phymatotrichum omnivorum* is more closely related serologically to the puffballs than to any of the other forms represented in these tests.

The Complement Fixation Test.—In the complement fixation tests the sera of rabbits No. 2 and No. 3 yielded complement fixing antibodies in such low dilutions that they could not be used. The serum of rabbit No. 1 contained complement fixing antibodies in an antiserum dilution of 1:160, when tested against the homologous antigen. Consequently, this serum was employed in testing the relations of the various antigens to the antigen of the cotton-root-rot fungus. The results of these tests are shown in table 5. One may observe from this table that the puffball forms, viz., *Lycoperdon gemmatum*, *Secotium acuminatum*, and *Calvatia cyathiformis* again appear to be more nearly related to *Phymatotrichum omnivorum* than do any of the other genera tested.

By comparing the results in table 5 with those of table 6, one may readily observe that some of the species do not assume the same ranks in the 2 tests.

This is relatively inconsequential and is to be expected when use is made of 2 different sorts of tests. Indeed, 2 tests of the same sort will often present minor discrepancies. The most important and significant feature of these tests is that in both cases the 3 puffballs are ranked closer to the *Phymatotrichum omnivorum* than are the other genera. This evidence should serve to establish, at least presumptively, the serologic relation of the cotton-root-rot fungus.

THE UNIVERSITY OF TEXAS

COTTON ROOT ROT INVESTIGATION AND RESEARCH

DEPARTMENT OF BOTANY AND BACTERIOLOGY

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A WHITE ROOT ROT OF APPLE TREES CAUSED BY *CORTICIUM GALACTINUM*

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INTRODUCTION

Very frequently, in the investigations of apple root diseases, white fungi have been found associated with dead or dying roots. These fungi usually have proved to be saprophytes growing on dead or nearly dead roots. In 1932, however, a white fungus that appeared to be an active pathogen was observed growing on the roots of an apple tree at Heards, Virginia. Since that time studies on this fungus have established its active parasitism on apple-tree roots and its identity as *Corticium galactinum* (Fr.) Burt.

Von Schrenk¹ in 1902 published a brief note on a root rot, apparently identical with this disease, caused by *Thelephora galactina* Fr. (now a synonym of *Corticium galactinum* (Fr.) Burt). In 1909, Von Schrenk and Spaulding² stated that this fungus occurs commonly as a root parasite of oaks in various parts of the Ozark Mountains and that it spreads from oaks to fruit trees when the latter are planted on recently cleared land. No reference in the literature to this disease has been found since the publication of these two short notes.

¹ Von Schrenk, H. A root rot of apple trees caused by *Thelephora galactina* Fr. *Bot. Gaz.* 34: 65. 1902.

² Von Schrenk, H. and P. Spaulding. Diseases of deciduous forest trees. U. S. Dept. Agr., Bur. Plant Ind. Bull. 149. 1909.

SYMPTOMS

Usually the disease starts at the collar or on larger roots and advances rapidly outward on smaller roots. Often the killing is so rapid as to kill the larger roots near the collar and girdle the tree while the distal portion of the roots may be still alive. The killing is usually so rapid and complete that the presence of the disease in the roots is manifest only in the top by the sudden death of the whole tree. On the other hand, the action of some slower developing root-disease fungi, such as *Xylaria*, is manifested in the top by weak limbs on the side of the tree above the diseased roots.

In the first stage of attack there is a growth of white hyphal strands on the surface of the root. As the fungus grows this surface layer becomes thicker and thicker till a dense web of mycelium covers the surface of the root (Fig. 2, A and C). The fungus meanwhile gradually penetrates the epidermis, then the cortex and the cambium, and finally the wood, causing a white wood rot.

The cambium is not uniformly killed. This is shown by the zonate spots on the wood where the bark has been removed (Fig. 4, B). In some instances the margins of the spots are surrounded by incipient callus formation about the areas of killed cambium. In other cases where the host tissue surrounding the killed area is still more active, growth and enlargement take place, which result in a very peculiar and distinctive-looking root bearing pits and bumps over the surface (Fig. 4, A). Sometimes there is another manifestation of the disease resulting in a general hypertrophy of the root at the junction of the diseased and healthy areas.

DISTRIBUTION

The disease was observed at Heard's, in 1932, and since that time other trees in the same orchard have been killed. Affected trees have also been observed in the fruit regions near Middletown, Luray, and Leesburg, Va., Beltsville, Md., Greenville, Tenn., Bridgeville, Del., and Bedford, Ind. Von Schrenk³ noted that root rots of apple trees were abundant in Kentucky, Missouri, Illinois, Arkansas, Oklahoma, and West Virginia, and considered *Thelephora galactina* one of the chief causes. The disease has been observed in a relatively small number of orchards but distributed over a wide area. Its presence has been confined to orchards that were set on newly cleared land or orchards in close proximity to a woods.

At various times since 1932, field surveys of root rots of apple trees have been made in many of the fruit regions in the eastern part of this country to study the types of root disturbances and their relative prevalence in the different regions. Very little survey work has been done, however, in the Ozark region where the disease was first reported. This present work has not been intensive enough in any one region to determine as fully as desired the prevalence, severity, and relation of the disease to environment. Reports from orchardists and oral reports from other investigators indicate

³ See footnote 1.

that a thorough survey would show it to be more common and important than is realized at present.

PATHOGENICITY

Observations of diseased orchards and the gaps resulting from the death of trees indicate that the disease is more serious after they are 14 to 18 years old than in the case of younger trees. In one case a 3-year-old tree standing in the nursery was successfully inoculated in 1937. When inspected 1 year later no definite necrosis was found but the bark surface was abnormally rough and there was cortical thickening, as though cork tissue had effectively cut off and completely healed an extensive area nearly encircling the root where the fungus had initiated infection. On the other hand, no bearing tree has been observed to recover from infection. Other inoculation experiments and also field observations indicate that apple trees are more susceptible to this root disease after they begin heavy bearing than before. This young bearing stage in orchard trees has also been found to be an especially susceptible period for winter injury and for such diseases as *Phytophthora collar blight* and *Xylaria root rot*.

Repeated cases have been found showing the ability of this disease to kill trees very rapidly. In the summer of 1936, some trees that had made good terminal growth the previous year died while they were carrying a good load of fruit. In another case an apparently vigorous 15-year-old tree was well-laden with mature and marketable fruit the latter part of August, even though it was completely girdled by the fungus. In both instances the action of the pathogen was so rapid that the appearance of the tops gave little indication of the diseased condition of the roots until the trees died.

The pathogenicity of this fungus also was studied by isolation and inoculation work. Cultures used for inoculation were obtained from spores and from isolations made from diseased apple tree roots taken from the margin of healthy tissue. Work with this fungus has demonstrated what has also been observed with other root rot organisms—that the quantity and types of inoculum used influence infection. The best type of inoculum was obtained by growing a pure culture of the fungus a month or two on short sections of heat-sterilized apple twigs. Where the tree was inoculated *in situ* root tissue was exposed by removing the soil, the inoculum placed against the uninjured root and the soil immediately replaced. The trees were not further disturbed until the end of the growing season, when they were inspected for infection. When trees were inoculated while in storage the inoculum was held against the root with a rubber band.

Young trees growing in the nursery were successfully inoculated with naturally infected apple roots and also with a pure culture of the fungus. In September, 1936, naturally infected roots were placed beside 10 3-year-old trees in a nursery row, and a year later 5 trees were infected, showing typical signs of the disease. In 1937 and 1938 4 inoculation experiments were made on young trees growing *in situ*, using a pure culture of apple twig inoculum. In one experiment a culture obtained from spores was used and in the other

3 the cultures were from diseased apple roots. All 4 cultures produced the disease, averaging 18 per cent infection in 49 inoculations. A like number of checks were uninfected.

The pathogenicity of the fungus with respect to dug apple trees also was studied by inoculation experiments. In April, 1937, 40 1-year-old seedlings from the nursery storage were inoculated by binding without wounding a twig culture of the fungus with a rubber band to the main root. The trees were then immediately planted. An equal number from the same lot of trees were planted without inoculation, to serve as checks. By July 20 the roots of all the inoculated trees were dead, while the checks were unaffected and grew in a normal manner. From the infected roots was isolated a fungus that appeared to be identical with the fungus used for inoculation.

In March 1938, apple seedlings were divided into 3 comparable lots of 25 trees and each lot inoculated with 1 of 3 different spore isolates. These seedlings were then stored in a cool cellar in peat, and, on June 1, examination showed an average of 95 per cent infection on all 3 lots. Root cuttings inoculated and planted in February, 1938, showed 9 infections in 18 inoculations by October of the same year, while the noninoculated checks showed no infection.

These experiments indicate that young trees, as they grow in the nursery, may be successfully inoculated with a pure culture of the pathogen but the percentage and degree of infection obtained on such trees have been very much less than on stored nursery trees or trees disturbed by digging.

THE FRUITING STAGE OF THE FUNGUS

According to Burt⁴ *Corticium galactinum* (Fr.) Burt, fruits on a variety of substrata, including wood of both coniferous and broadleaf species, is widely distributed in North America, and is present in the West Indies and Japan. It seems reasonable to expect the species so defined to be composed of several physiologic strains, but the writers have studied only the form on apple roots.⁵ It is hoped that a consideration of the broader aspects of the species will eventually be undertaken to determine to what extent native trees and shrubs, also, when growing under natural conditions, are affected by it.

In general, the fruiting on apple roots and stumps agrees fairly well with the description given by Burt, except that he does not mention the conspicuous slightly protruding paraphyses (Fig. 1, A), which have been observed in all our specimens. Most sporophores collected do not contain a distinct basidial layer; therefore, these paraphyses constitute the most characteristic feature of the species. Since no illustrations of the fungus were given in Burt's monograph of the genus, drawings are given showing some characters of the organism dealt with in this paper.

⁴ Burt, E. A. The Thelephoraceae of North America XV. Ann. Missouri Bot. Gard. 13: 173-354.

⁵ A sporophore from an apple stump was sent to L. O. Overholts, who identified it as *Corticium galactinum*.

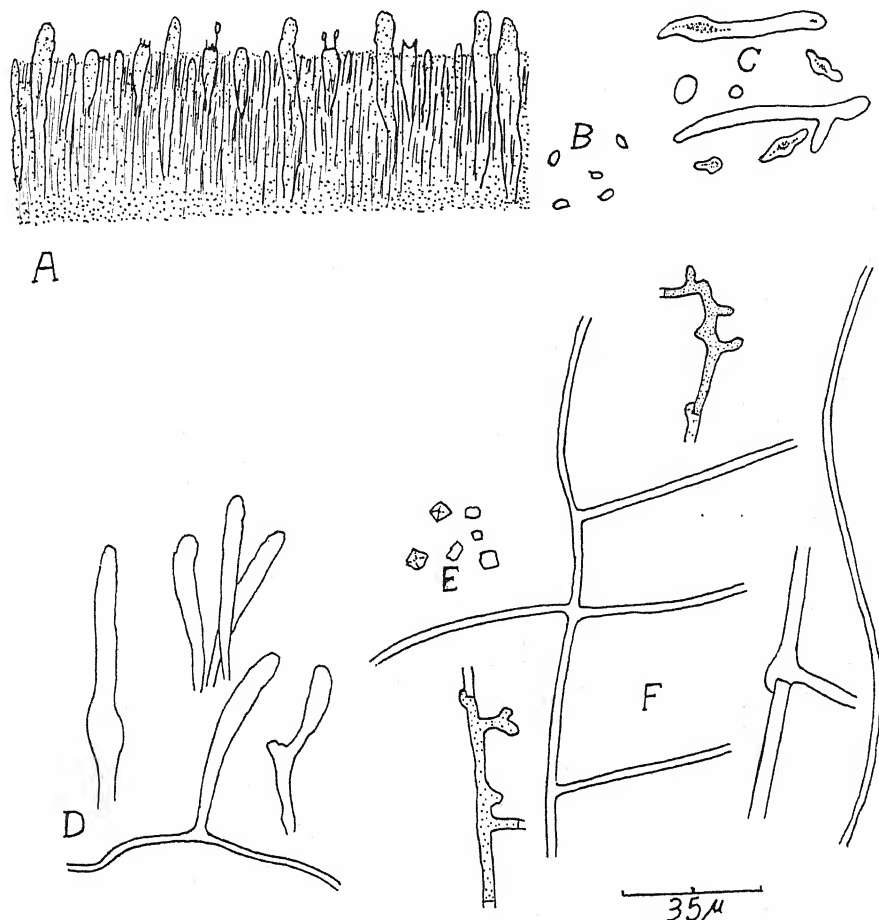


FIG. 1. *Corticium galactinum*. A. Sketch of a section through the hymenium. B. Basidiospores. C. Germinating basidiospores. D. Paraphysis-like bodies from a culture. E. Crystals from pure cultures on malt agar. F. Hyphae from pure cultures.

The dense white to light-cream buff layer of mycelium, on which the hymenium develops when conditions are favorable, may persist over a period of several years on old apple stumps and roots. The basidia apparently develop during damp weather in summer or fall and soon collapse. They are entirely absent under dry conditions. In fact, sections from fresh sporophores, which gave good spore prints, contained very few mature basidia. Sporophores usually are formed in soil cavities about rocks or on roots (Fig. 2, D), but during favorable growing conditions the fungus may grow out over the surrounding soil and debris where it fruits in abundance. It may be that the habit of fruiting on soil and surrounding debris is responsible for its having been reported on such a variety of substrata.

The dry fruiting layer is white to light buff; but in a damp and sporulating condition it has a slightly waxy appearance on the surface and, in

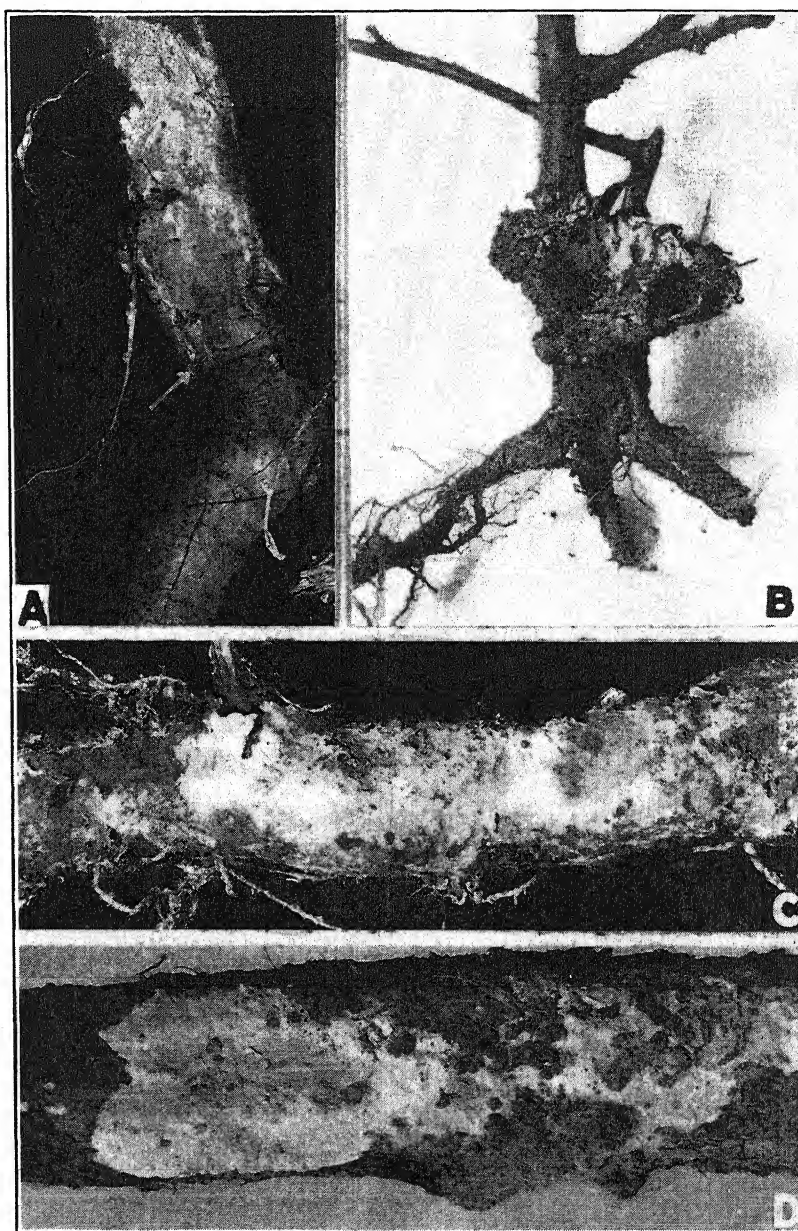


FIG. 2. A. Mycelium of *Corticium galactinum* on the surface of an apple root. This photograph also shows the sharp demarcation between sound and diseased tissue indicated by the arrow. B. Infected blackberry showing hymenium of *C. galactinum* around the collar and mycelium on the roots. C. Infected apple root covered with thick web of mycelium. D. Hymenial layer on an old apple root.

color, ranges from light buff to ochraceous buff.⁶ Von Schrenk⁷ states that the fruiting bodies are "bright red orange leathery sheets" but our observation indicates the hymenium is not so highly colored. The thickness of the fruiting body presumably depends upon its age; specimens several years old have been observed with fruiting bodies up to 500 μ thick.

The structure of the sporophore is not very distinctive, except for the paraphyses, which project up to 12 μ above the surface of the hymenium and penetrate into the subhymenium to a depth of about 30 to 40 μ . Basidia with immature spores attached may be found occasionally in sections of fruiting sporophores (Fig. 1, A), but usually even young basidia are difficult to find. Spores are not abundant on dry specimens but are easily obtained from good fresh material. A spore print can be obtained by placing under a bell jar or other closed container such sporophores with the hymenium downward. Also, small sections 1 or 2 cm. square, cut from the hymenial layer and suspended over Petri dishes containing nutrient agar, will deposit spores in great abundance for a period of 12 to 18 hours if left in a moist condition. The mature basidiospores are ovoid, hyaline, smooth, and 3-4 by 2-3 μ (Fig. 1, B) in size.

HOST RELATIONS

The white root-rot organism will attack the roots of plants other than the apple, but the observations to date have included only plants growing in the vicinity of the focus of inoculum of diseased apple trees, or, in one case, an oak stump. The pathogen has been found growing abundantly on the roots of blackberry (*Rubus allegheniensis* Porter), dewberry (*Rubus flagellaris* Willd.), Japanese wineberry (*Rubus phoenicolaris* Maxim.), dogwood (*Cornus florida* L.), sumac (*Rhus glabra* L.), and white campion (*Lychnis alba* Mill.). More information is needed concerning the susceptibility of various species.

One instance has recently been found in which ornamental plantings have been affected by this disease. In a yard near Hyattsville, Maryland, two disease spots were observed in which a young holly tree, a dogwood, and two Kalmia bushes were removed because of this disease. Near the spots where these plants died was an oak stump on which *Corticium* was growing and producing fruiting bodies typical of *Corticium galactinum*. This and other observations indicate that when ornamental shrub plantings are made in newly cleared land, white root rot may become a problem.

THE FUNGUS IN CULTURE

Isolation and Spore Germination

Spores deposited directly from segments of the hymenium, as described above, on Petri dishes containing Difco cornmeal agar or malt agar germinated in 10 to 16 hours (room temperature of about 26° C.) (Fig. 1, C.)

⁶ Ridgway, R. Color Standards and Color Nomenclature, 43 pp. Washington, D. C. 1912.

⁷ See footnote 1.

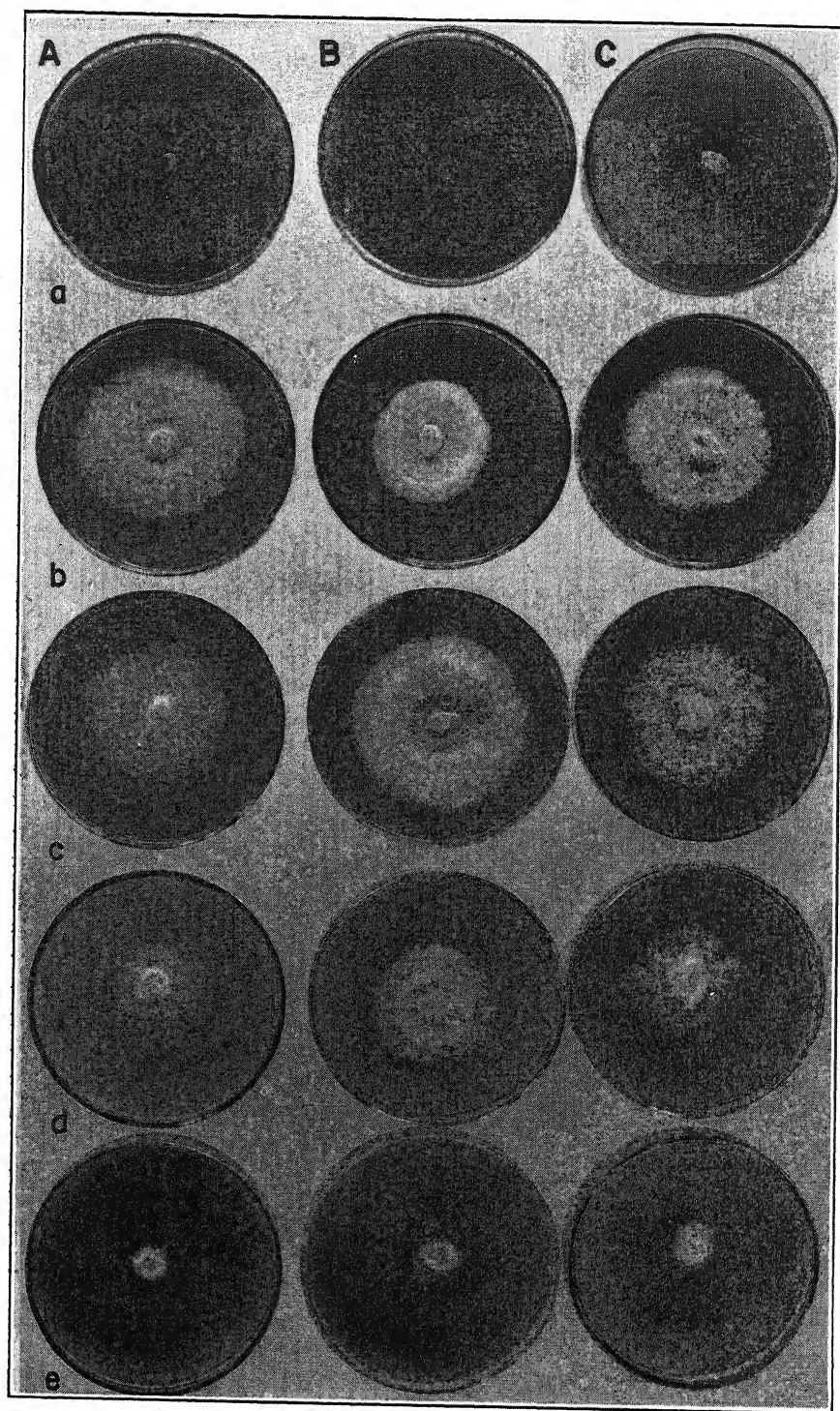


FIG. 3. Eight-day-old cultures of *Corticium galactinum* grown in constant temperature chambers. A. Culture from diseased apple root. B. and C. Basidiospore cultures. a. 34° C., b. 31° C., c. 25° C., d. 20° C., e. 15° C.

and a large proportion of the spores were viable. Cultures were obtained also from recently formed lesions on living roots and were similar in general growth characteristics, as well as in microscopic characters, to those from spores.

Temperature Relations

Four cultures obtained from spores and 2 from diseased roots were grown on 2.5 per cent malt-agar medium in the dark at various constant temperatures. The average diameters of mycelial mats for all cultures, including culture 7138-S after 1 day at ordinary room temperature followed by 7 days in the constant temperature chambers, were as follows: At 10° C., 16 mm.; at 15°, 22 mm.; at 20°, 41 mm.; at 25°, 61 mm.; at 31°, 45 mm.; at 34°, trace; and at 40°, no growth (Fig. 3). Growth rate at the various temperatures was fairly uniform for all cultures except spore culture 71383-S, which had a mat diameter of only 26 mm., at 20°; 41 mm., at 25°; and 33 mm., at 31°. The optimum temperature for growth of all cultures used

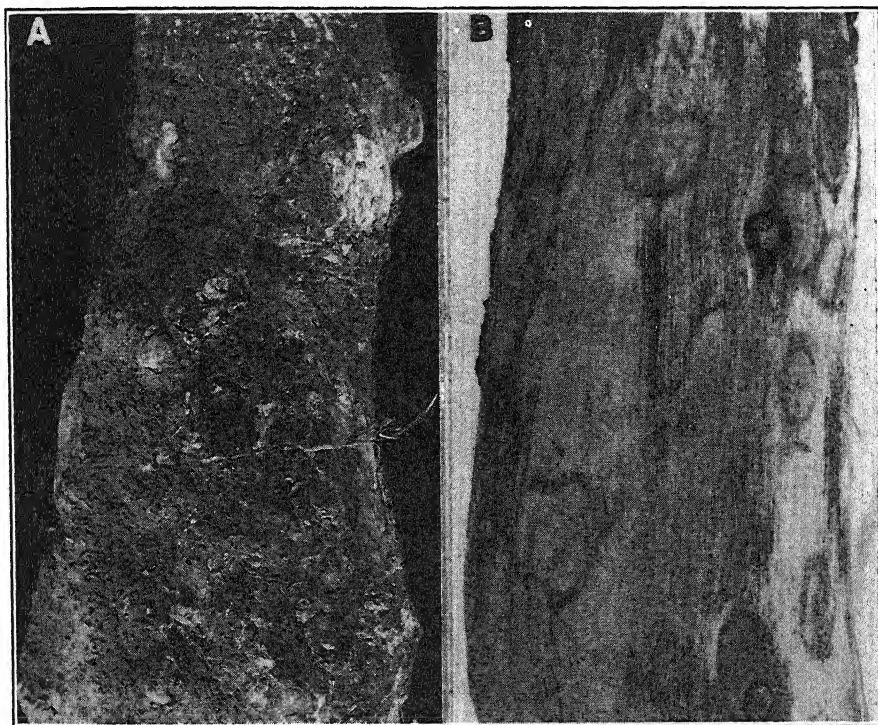


FIG. 4. A. Distorted apple root illustrating partial recovery. The depressions shown at the margin of the photograph indicate areas that were killed by *Corticium galactinum*. B. Dead apple root with bark removed to show the characteristic rings formed around areas of initial killing. The entire root was killed before callus tissue was formed.

was between 25° and 31° and maximum temperature was apparently slightly above 34°.

SUMMARY AND CONCLUSIONS

The paper describes a white root rot of apple trees that escaped the notice of pathologists from its discovery in 1902 until 1932. The fungus isolated from diseased roots has been identified as *Corticium galactinum* by comparing it with cultures obtained from sporophores of *C. galactinum* from other sources. The cultural characteristics of the fungus are described.

While the disease has been found in relatively few orchards scattered over Delaware, Virginia, Maryland, Tennessee, and Indiana, more surveys will probably show its distribution to be much more widespread than is now known.

Since the disease has as yet been observed only in orchards set on new land or adjacent to woods, it seems probable that new land furnishes conditions favorable for the supplying and the maintenance of the inoculum. This observation, together with the virulence of the disease in certain orchards, justified the conclusion that consideration should be given to this disease in choosing an orchard site.

The organism is very destructive in its attack. The spread from tree to tree seems slow but after infection is established killing is very rapid. A slow advance of the disease has been noted in all the orchards under observation.

Young apple trees were successfully inoculated with the fungus described in the paper. Undisturbed nursery trees were much less susceptible to the disease than dug trees. Trees of bearing age showed greater susceptibility to the disease than younger ones.

Several other species of plants when growing in proximity to diseased apple trees or oak stumps became infected.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES
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APPLE DIEBACK IN CALIFORNIA¹

P. A. ARK AND H. EARL THOMAS

(Accepted for publication September 9, 1939)

INTRODUCTION

Dying back of apple branches usually is accompanied by relatively few distinctive characteristics. The disorder here presented is commonly prevalent in the Sebastopol area of Sonoma County, California, one of the two leading apple districts of the State, and has been seen in at least one other county (Eldorado). Trees may develop severe symptoms for the first time at any age from 1 or 2 up to 25 years or more (Fig. 1, A, D). Depending on the severity, the buds may die without starting growth, may push out a few

¹Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

small leaves and then die, or may slowly develop a sparse foliage of small, narrow leaves (Fig. 1, B). When the buds die early, the bark above the ground may break down in a few weeks, often with a strong odor, which has given rise locally to the term sour sap. In somewhat less severe cases, the bark becomes densely covered with protuberances, each underlaid by necrotic tissue in the interior of the bark. These may break down later, often with more or less concentric marking. This bark symptom falls in the general class variously called measles, target canker, etc.

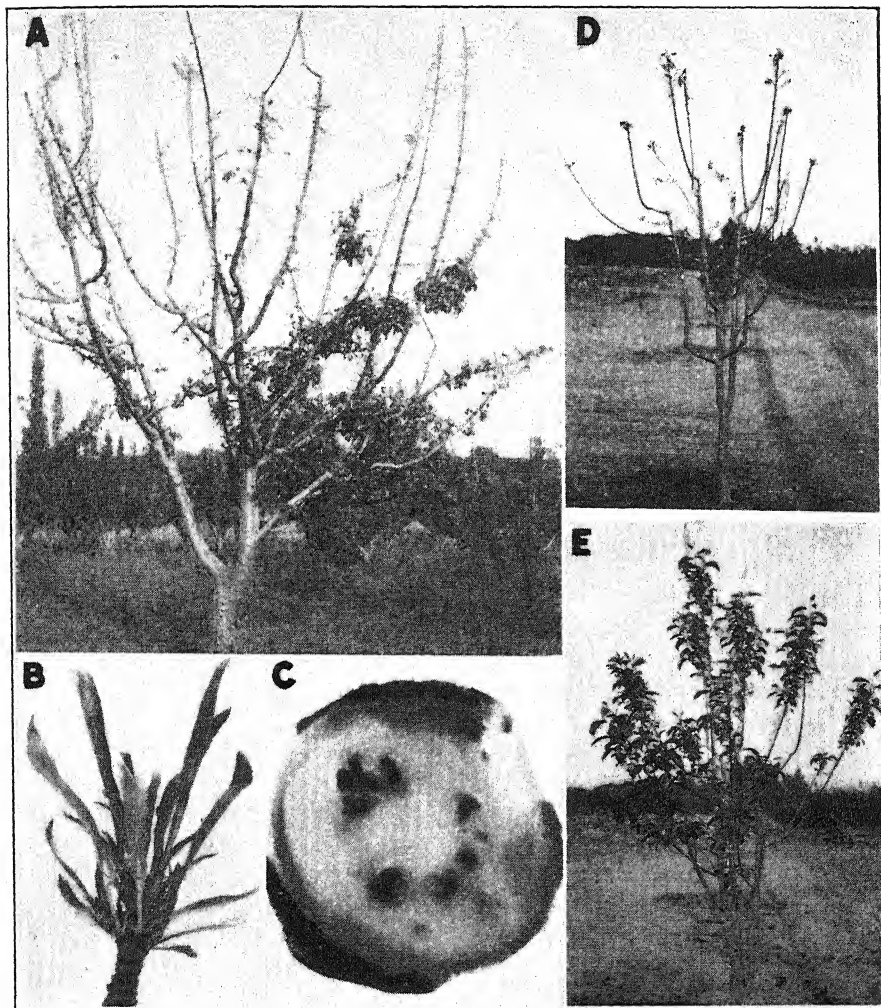


FIG. 1. A. Gravenstein apple tree showing dieback symptoms. B. Dieback disorder on a Spitzenberg apple tree. Opened buds had very narrow leaves and short petioles. C. Cross section at the base of a bud that failed to develop in the spring and remained dormant all summer. Note the necrotic pockets in the region of the vascular elements. X15. D. Young Rome Beauty tree showing severely affected buds. E. Apparently healthy Rome Beauty tree adjacent to that in D photographed at the same time, May 5, 1930.

Much of the disorder is found in Gravenstein, which is by far the leading variety of the Sebastopol area, but Spitzenberg and Wagener seem to be distinctly more susceptible. Delicious is particularly susceptible to the measles type of trouble here as elsewhere. Pears are affected with symptoms similar to those of the apple.

Specific fruit symptoms were seen in 1937, apparently for the first time in California; though the dieback phase of the disease has been under observation for 30 years or more (19). These fruit symptoms (Fig. 2) are in the main typical of the cork and drouth spot, adequately described by Mix (17) and others (1, 2, 3, 4, 13, 14, 18), and appeared following heavy winter rains and high early summer temperatures.

Rosette or little leaf, which also occurs in the Sebastopol area (6), is distinguishable with difficulty, if at all, by symptoms from the dieback disease under consideration. Usually, however, on rosette trees the tips of many branches remain alive until late stages with distinctly thickened shoots and very short internodes near the tips resulting in the compact tufts

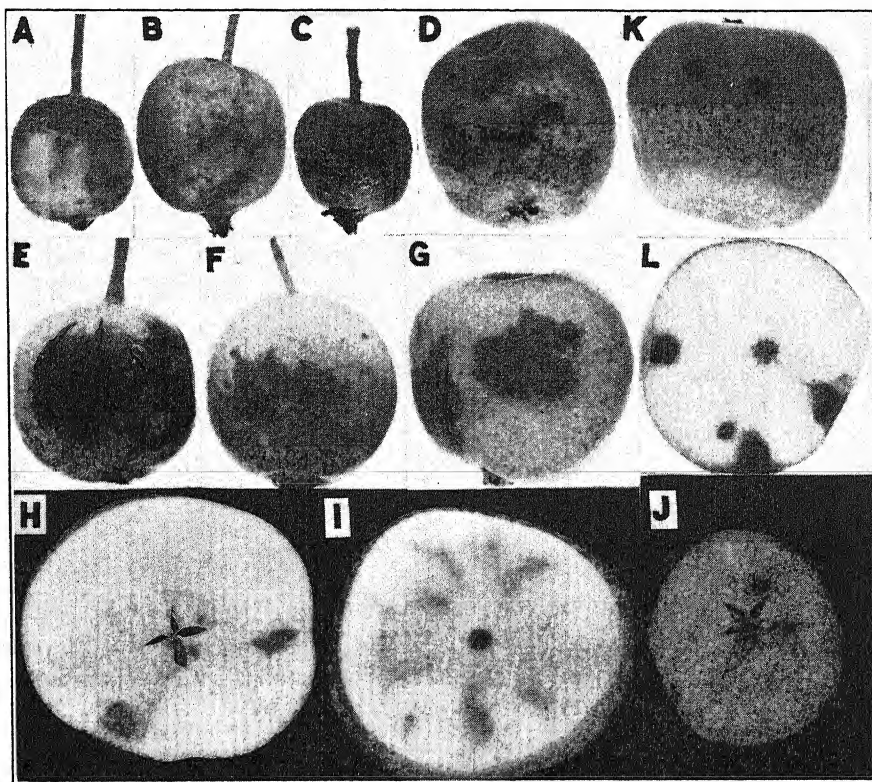


FIG. 2. A-D. Symptoms of drouth spot on fruits of Spitzenberg apples. E-G. Symptoms on fruits of Red Rome apple. Note the fingerprint pattern in F and G. H-J. Internal cork on a Gravenstein apple. H. Internal light-brown areas, which usually occur between the large vascular elements. I and J. A diffused type of cork. K. External appearance of fruit from a Jonathan apple tree affected with internal cork. Note pitting of the fruit. L. Cross section of the fruit shown in K. All $\times \frac{1}{4}$.

of leaves, which gave rise to the name. The petioles here are often almost entirely suppressed, while on dieback trees they usually reach a length of at least half an inch or so. No specific fruit symptoms seem to have been found on rosette trees.

SOIL OF AFFECTED ORCHARDS

Orchards in which dieback occurs are planted in Goldridge fine sandy loam soil. The subsoil of the better orchards is typically a pervious clay, while in many, if not all, of the affected areas the subsurface strata contain considerable cementing material. This soil is distinctly acid, relatively low in fertility, especially K and P (12), and is dependent upon winter rainfall (15 to 40 inches) for its water supply. Glass-electrode determination of pH on soils from very severely affected orchards ranged from 4.95 to 5.45 (top soil). The application of chicken manure is commonly practiced among the apple growers in this district.

EXPERIMENTAL

Bacteriological Approach

Numerous attempts to isolate a pathogenic organism were made by different individuals at the California station at different times, which yielded only negative results.

The idea that some toxic material was being liberated by soil microorganisms or was being formed by some unfavorable soil condition has been entertained for some time. It was observed that soil samples from affected orchards, when artificially waterlogged and subsequently incubated anaerobically, always had a putrid odor, were dirty blue, and the soil filtrate proved toxic to barley seedlings and to young, tender apple shoots. Apple seedlings, grown in artificially waterlogged soil that had been incubated anaerobically in the laboratory for a month, developed very poorly in comparison with those grown in soil that was not waterlogged but was also incubated under anaerobic conditions for a month. However, the plants in the treated lot eventually recovered unless the soil was constantly waterlogged. It is quite possible that microorganisms may cause the dieback condition by absorbing from the soil large amounts of elements that are indispensable for the normal development of higher plants. By Cholodny's method (7), applied to the rhizosphere of diseased apple trees, it appeared that long and short bacterial rods predominated over fungi and *Actinomyces*, while, in the rhizosphere of healthy (unaffected) trees, fungi and *Actinomyces* are more abundant. Sterilization of soil from a dieback orchard either by steam or with formaldehyde failed to produce symptoms of boron deficiency in annual plants, symptoms appearing after inoculation of sterilized soil with a small quantity of nonsterilized soil and subsequent incubation. More detailed treatment of this phase will appear in a later publication.

Relation of Boron to Dieback

In the spring of 1937, in orchards where dieback of apple was severe, morning-glory plants were found with dead or dying growing points, burning of tips of the leaves, and abscission of blossoms. Also, one sunflower plant appeared stunted in growth, with browning and malformation of the developing leaves. The plants were taken to the greenhouse for observation, and it soon became apparent that these plants were suffering from boron deficiency. By adding 1 mg. of boric acid to a gallon of the same orchard soil in which these plants were found, normal growth of the morning-glory and sunflower plants was resumed.

One-gallon tin cans, painted on the inside with asphaltum, were filled with either top soil or subsoil taken at a depth of 2 feet. To one series of 10 cans in each case, 1 mg. of boracic acid was applied at the time of planting, while the second series had no treatment. All sugar beet, sunflower, nasturtium, and lettuce plants grown in the nontreated soil developed characteristic symptoms of boron deficiency, while in the treated lots all plants developed normally. The low availability of boron in soils of affected apple orchards, as indicated by these tests, suggested that application of boron-containing compounds to the diseased trees might prove beneficial. The use of boron as a corrective for similar conditions in apples has been made by a number of investigators (3, 5, 15, 16, 18).

It is interesting to note that the total sugar analysis of the leaves from the diseased and healthy apple trees employing Hassid's (9) method showed 5.56 per cent in leaves and 2.6 per cent in twigs for diseased and 3.8 per cent and 3.04 per cent for healthy trees. This seems to be in accordance with the statement of Haas and Klotz (8) that sugars accumulate in the leaves whenever boron is omitted.

Attempts to produce symptoms of boron deficiency in deciduous fruit trees were made by growing apple, peach, and apricot plants in subsoil obtained from affected apple orchards and watering them with distilled water. Some of these plants were seedlings and others were commercial varieties grafted onto seedlings. Only apricot developed symptoms of boron deficiency, as described by Hoagland, Chandler, and Hibbard (11), this being corrected by giving 0.1 g. boric acid to each jar containing 10 kg. of nonsterilized subsoil.

Field application of boron to diseased trees was started in the winter of 1936 and the spring of 1937. Boric acid or borax was applied to the trees by boring a hole in a large branch or in a trunk and packing it with the material. These treatments produced no striking difference in the treated trees in comparison with nontreated trees.

In the fall of 1937 and in January and February of 1938, 35 trees in one orchard (Gravenstein, Delicious, Jonathan, and Rome Beauty) having dieback of branches, cork, and measles, and 20 trees in another orchard (predominately Spitzenberg) showing typical symptoms of drought spot and dieback, were treated by broadcasting borax on the soil around the trees

within a radius of 3 feet from the trunk. In one of the treated orchards, the trees appeared more thrifty the following year than the nontreated. One large Gravenstein tree with very severe dieback symptoms was greatly improved the year after receiving 10 pounds of borax, and still better in the second season. Mild dieback and severe and moderate cork symptoms of 8-year-old Jonathan trees were no longer visible after 2 pounds of borax had been applied as dressing, while corresponding checks were badly affected. The fruit on large Gravenstein apple trees still had cork when treated with $\frac{1}{2}$, 1, 2, 3, and 4 lb. of borax. Cork was considerably reduced in such trees treated with 5 lb. or more of borax.

In the other orchard, where drought spot on fruit was abundant the season prior to treatment, no conclusion could be reached, since fruit symptoms were lacking and the general appearance was similar for all trees.

Delicious trees, affected with measles in the first-mentioned orchard, seemingly were benefited by the boron treatment, since they made good growth and did not show the protuberances and cracking of bark on the new growth.

On the basis of somewhat limited treatments, there seems to be a definite indication of beneficial effects by applying boron to trees affected with cork and measles and to some trees showing dieback symptoms only.

RELATION OF POTASSIUM TO DIEBACK

Chemical analyses of soils in affected orchards in the Sebastopol area have shown a very low potassium availability, according to the work of Hoagland and Martin (12). Hill and Davis (10), in Canada, found cork in orchards grown on soils with low available potassium. In 1937, McLarty, Wilcox, and Woodbridge (16), in referring to orchards with dieback, stated that "heavy applications of potash have in some cases materially lessened the disease symptoms." At the suggestion of W. H. Chandler, a few severely affected trees were treated with heavy applications of potassium sulphate (approximately 25 and 75 lb. per tree) early in 1937. The treated trees improved considerably in one orchard and only slightly in another. It is possible that the boron in these rather large amounts of potassium sulphate may have produced the observed effect. The experience thus far on the whole, however, suggests that in some, if not all, of these orchards, more than one element will be required to completely cure the affected trees. At least it seems to be true that if boron alone is to be completely effective it must be used in much larger amounts than is required in other areas. More extensive experiments are under way including combination treatments with boron, potassium, and other materials.

SUMMARY

Dieback of apple trees, often accompanied by a type of "measles" and occasionally by cork and drouth spot of fruit, is prevalent in the Sebastopol area of California.

The soil is distinctly acid and low in available nutrients, notably potassium.

Such annual plants as nasturtium, sugar beet, and sunflower, grown in soil from affected orchards, developed boron-deficiency symptoms curable by addition of small amounts of borax or boric acid to the soil.

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METHODS OF VALUE IN BREEDING AUSTRIAN WINTER FIELD PEAS FOR DISEASE RESISTANCE IN THE SOUTH¹

J. L. WEIMER

(Accepted for publication Sept. 25, 1939)

INTRODUCTION

Soon after the writer began the study of the diseases of Austrian Winter field peas (*Pisum arvense* L.) at the Georgia Agricultural Experiment Station, three major difficulties were encountered. In the first place, almost none of the varieties of peas (*P. arvense* and *P. sativum*), except the Austrian Winter, survived the usual winter temperatures. This made it impractical to test any large number of varieties of peas for disease resistance under field conditions during the winter. Plants from seed sown in the spring were killed very early by insects and diseases; hence, changing the planting time from autumn to spring was of no assistance. Even though some varieties survived the milder winters, the plants usually succumbed to diseases or insects, or both, before seed matured. Furthermore, nature could not be depended on to produce an epiphytotic of the diseases being studied, so that the relative resistance of varieties, plants, or hybrids could be determined. This made it necessary to use some method of artificial inoculation.

Solutions for certain of these difficulties have been found and are described in this paper. No claim is made that these methods are new, but rather that they have been useful in solving the problems under consideration, and it is hoped will be of assistance to others having similar problems.

ELECTRIC HOTBED

Since so few varieties of peas survived the winters at Experiment, Georgia, or even at Tifton, the following method for overcoming this difficulty was tested.

Two hundred feet of electrically heated and controlled hotbed in 50-ft. units, each 5½ ft. wide, were constructed (Fig. 1, A). The walls of the beds were made of pine boards and were 1 ft. high on one side and 2 ft. on the other. The high side was covered with heavy building paper primarily as protection over the crack between the boards and at the corners and around knot holes. Instead of sash, the top was covered with a heavy cotton sheeting. The covers were used only on nights when there was danger of frost and during the coldest days. Thus, for all practical purposes, the plants were exposed to field conditions, except during the coldest weather.

¹ Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 65, Journal Series, Georgia Agricultural Experiment Station.

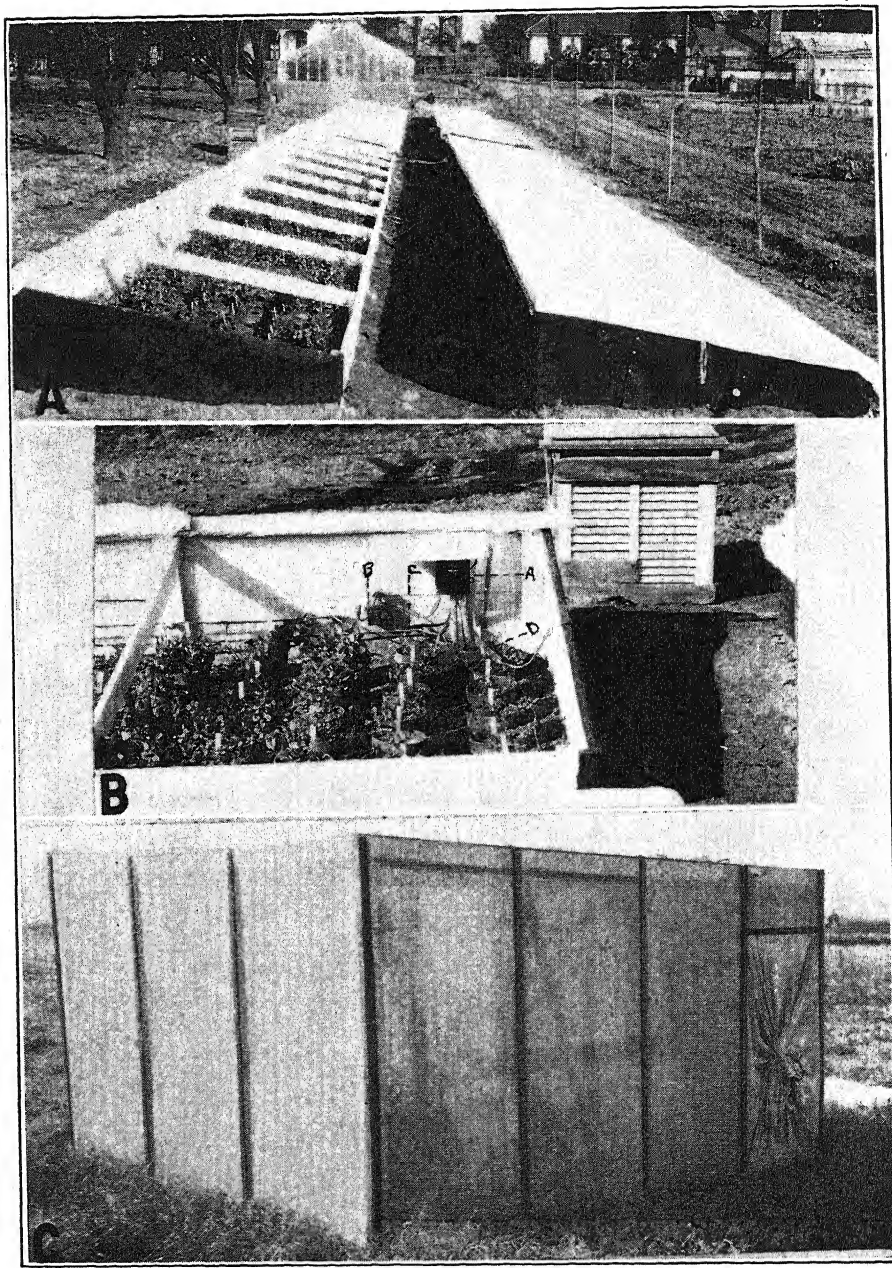


FIG. 1. A. Four hotbeds each 50 feet long by $5\frac{1}{2}$ feet wide, used to grow peas for testing for disease resistance. First bed on the left has the cloth cover rolled back; the other 3 have the covers in place. Several details of construction are shown here and also in B. B. A cut-off switch A (inked letters), thermostat B, and thermostat bulb C are shown. The thermostat bulb was suspended over a pot. The thermograph bulb D was placed over a pot also so as to obtain the temperature to which the bases of the plants were actually exposed. C. A cloth-covered house in which a good seed crop has been obtained from a number of different varieties of peas.

The electric cable was placed on the surface of the soil and looped back and forth across the bed at distances convenient for spacing rows of pots, in which the plants grew. Thus the cable lay along two sides of every pot (Fig. 2). At first, 360 ft. of cable were used for each 50 ft. of hotbed, but later 120 ft. more were added to 2 of the beds. This additional cable was not needed, since the lowest official temperature for the winter was only 19° F. One thermostat was used for each bed (Fig. 1, B). Two hundred and twenty-volt current was utilized. The thermostats were set so that the heat was turned on at about 35° F. The thermostat markings were not reliable, but by placing the bulbs in water at the desired temperature, the settings could be made readily. Soil-thermograph bulbs placed on the tops of pots showed the temperature to which the bases of the plants were subjected. None of the plants were frozen, excepting a few leaves, which had grown up against the cloth top. Judging from the thermograph records (Fig. 3), it seems probable that the plants would have survived a temperature several degrees lower, but the limit is not known.

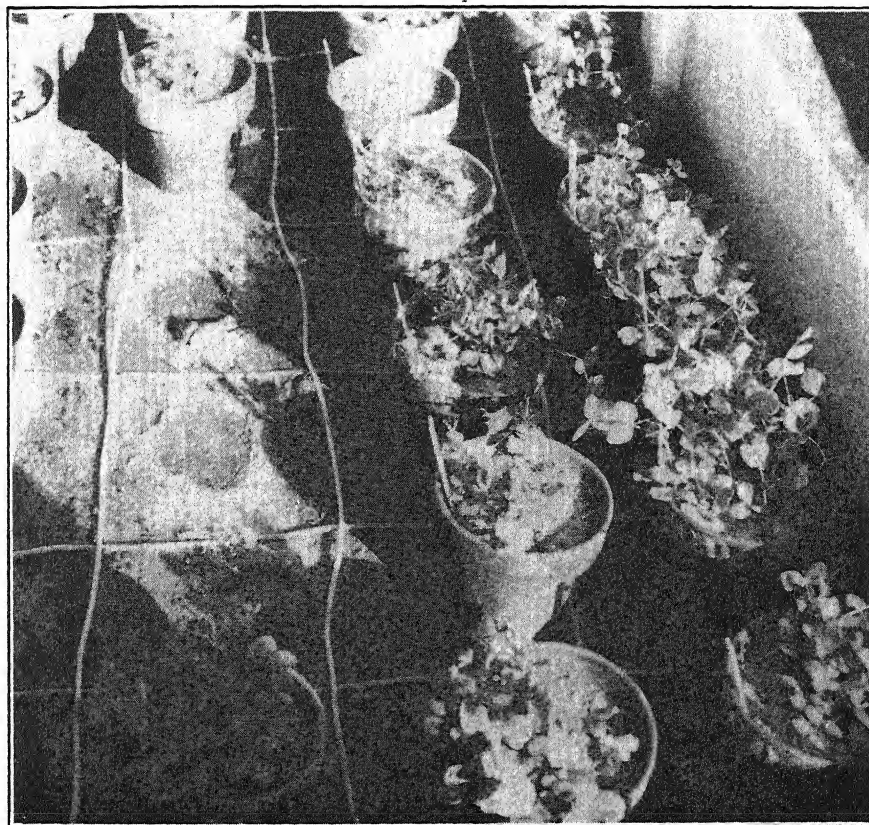


FIG. 2. A closer view of a section of one bed than shown in figure 1, A. Here the arrangement of the cable with respect to the pots is shown. The 3 longitudinal strands of cable were added after the season was partly over but were not needed.

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seed under field conditions at Experiment. Peas planted in the greenhouse and given additional light from 5 to 11 p.m. produced seed fairly well during the winter months. Greenhouse space, however, was insufficient to carry on the work on the scale desired; hence, a cloth-covered shelter was tried. It was known that similar shelters had been used successfully by other workers both for sweet and English peas. Austrian Winter peas, however, appear to be more exacting in their requirements for seed production than many of the field and garden varieties. A shelter $9 \times 12 \times 6$ ft. was constructed of 2×4 in. lumber. This frame was covered with a good grade of cheesecloth, which kept out all but the smallest insects. The door was of the ordinary type, such as is commonly used on insect cages (Fig. 1, C). Two 500-watt Mazda lamps with 18-in. R. L. M. Dome reflectors were so attached to the ceiling as to provide approximately equal illumination for all of the plants. These lights were turned on at 5 p.m. and off at 11 p.m.

Two crops of plants have been matured under cloth in this manner, one in the spring and one in the autumn. The spring crop consisted of plants grown from seed sown in pots in the autumn and held in the hotbeds until March 30, when they were removed to the cloth shelter. All varieties used had matured seed by June 6 or earlier. The autumn crop was planted in pots on July 29, 1938, and most varieties had matured their seed by November 15. Thus it is possible to grow two crops a year. In fact, the seed from the spring crop can be made to mature a seed crop early enough to be planted in the field or hotbed in the autumn.

METHOD OF INOCULATION

Early attempts to produce heavy infection of Austrian Winter peas with *Ascochyta pinodella* and *Mycosphaerella pinodes* under greenhouse conditions gave unsatisfactory results. A few small lesions were produced on the leaves and stems but these were not sufficiently numerous or large to appreciably damage the plants or enable one to judge their relative resistance.

The method now in use makes it possible to produce a severe epiphytotic of the diseases under outdoor conditions. The plants to be tested were potted and held in the hotbeds described above. Giant cultures of the fungi being tested were cultured on Austrian Winter field peas. The dry peas were soaked over night in water and boiled a few minutes the next morning. The water was then drained off and the peas were put in flasks or 2-quart fruit jars, plugged with cotton, and autoclaved at 18 pounds' pressure for 1 hour. Inoculation of the flasks was accomplished by adding several cc. of a suspension of spores from oat-agar cultures in sterile water and shaking the flasks vigorously every day or so to spread the inoculum. After about 10 days the pea medium was covered with spores. A heaping teaspoonful of these spore-coated peas was scattered over the surface of each pot beneath the young seedlings. The pots were then forcibly sprinkled with water so as to spatter the spores upon the young plants. After inoculation the cloth covers were kept over the hotbeds for 48 hours.

During this time the cloth was wet thoroughly several times to help maintain a high humidity in the bed. A second inoculation was made 11 days after the first. The seed was planted October 15, 1938, and the inoculations were made on November 11 and 22. By December 7 a few plants of the most susceptible varieties were nearly decayed off at or near the soil surface. At this time the amount of infection varied greatly; but by March 1, 1939, there was a severe epiphytotic in all the beds. The spores, splashed from the original inoculum as well as from the lesions fruiting on the stems and leaves during watering and by the rains, produced an increasingly heavy infection as the season progressed. Eventually, many plants were killed, large areas of the stems of others were blackened, and the leaves of most of the varieties were more or less severely spotted.

SUMMARY

The use of an electrically heated and controlled hotbed to prevent the freezing of English peas under test for resistance to *Ascochyta pinodella* and *Mycosphaerella pinodes* during the winter and spring months at Experiment, Georgia, is discussed.

Austrian Winter and many other field and garden peas do not set seed consistently or in satisfactory quantity in the field in many parts of the South. These crops will set seed out of doors under cheesecloth. At certain times of the year it is necessary to give the plants additional light. This was done by the use of 500-watt lamps alight from 5 to 11 p.m. each day. Two crops of seed a year can be grown by this method.

An epiphytotic of the diseases under investigation was produced by inoculating hotbed-grown potted plants with cultures of the pathogens.

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A DRY ROT OF POTATO STEMS CAUSED BY *FUSARIUM SOLANI*¹

ROBERT W. GOSS

(Accepted for publication August 19, 1939)

Potato plants showing symptoms unlike those of any of the common potato diseases were observed by the author in a test plot of Bliss Triumph potatoes located at Scottsbluff, Nebraska, in 1935. The most noticeable symptoms were yellowing and wilting of the foliage, sometimes preceded by a rosetting of the top and the formation of aerial tubers in the leaf axils. The underground stem always showed some rotting, varying from a slight basal rot to a complete dry rot characterized by a shredded appearance due to the remaining strands of woody tissues. In the less advanced stages the rot produced a softening of the pith, whereas the vascular cylinder ap-

¹Published with the approval of the Director as Paper No. 236, Journal Series, Nebraska Agricultural Experiment Station.

The writer is indebted to Mitrofan Afanasiev, James H. Jensen, and W. E. Deacon for assistance in this investigation.

peared woody and light yellow. The roots of affected plants were severely rotted. Neither stolons nor tubers were directly affected. These plants were at first thought to be affected with *Fusarium oxysporum* Schlecht., or even *F. solani* ((Mart.) App. and Wr.) var. *eumartii* (Carp.) Wr. (Syn. *F. eumartii*) with atypical symptoms resulting from conditions favoring a rapid decay of the stem, such as sometimes occurs in irrigated fields. Later in the same season, however, similarly affected plants were observed in non-irrigated fields under very dry conditions. Plants were received also from North Dakota showing similar symptoms.

A large number of isolations were made from the underground parts of many of these plants. Cultures of *Fusaria* were obtained from all specimens and 34 isolates that appeared to belong to sections *Elegans* and *Martiella*, were saved for further study. Only 1 isolation of *Fusarium solani* var. *eumartii* was obtained.

In preliminary pathogenicity tests 20 to 25 plants were inoculated with each isolate by stem punctures below the ground at about the time the plants were emerging. A similar number of plants were grown on inoculated² soil previously sterilized. Not all of these inoculations were made at the same time, even with a single organism; the tests extended over a 2-year period.

Twenty-four isolates belonging in Section *Elegans* were tested in the above manner and, of these, 13 were pathogenic, producing a wilt with vascular discoloration but not resembling the symptoms of the plants from which they were isolated. In culture all of these pathogenic isolates appeared morphologically similar to *Fusarium oxysporum* and the type of wilt was similar to that produced by known cultures of that species.

Ten isolates belonging to Section *Martiella* were similarly tested and 5 of these were pathogenic. All 5 pathogenic forms appeared morphologically similar to *Fusarium solani* (Mart.) App. and Wr. One of these cultures (No. 242) produced a disease resembling that observed in the field and further tests showed conclusively that this organism was capable of causing all of the symptoms of the disease and could be isolated in pure culture from the infected tissues of inoculated plants. During 4 successive years in the greenhouse, this organism has consistently produced a high percentage of infection, as shown in table 1. Infection resulted in a much more uniform expression of symptoms than had been obtained in previous work with other species of potato *Fusaria*. In order to test relative virulence and to study resulting symptoms, one series of comparable inoculations was made with cultures of *F. oxysporum*, *F. solani* var. *eumartii*, and *F. avenaceum*.³ The inoculations were made by stem punctures and by growing plants in inoculated soil previously sterilized and in similarly inoculated, unsterilized soil. The results are presented in table 1.

² The inoculum for the soil was prepared by growing the organism on sterile barley or in sterile soil to which a little bran and sugar were added. About 1 pint of inoculum was used for 25 lbs. of soil.

³ The cultures of *Fusarium oxysporum* and *F. solani* var. *eumartii* were obtained from L. L. Cash of the U. S. Department of Agriculture in February 1936. These cultures had proved pathogenic in previous greenhouse experiments. *F. avenaceum* was obtained from John G. McLean, University of Wisconsin, in September 1937.

TABLE 1.—Comparative pathogenicity tests with 4 species of *Fusarium* and a summary of all inoculations with *F. solani* No. 242

Cultures	Number of plants inoculated	Number healthy	Number questionable	Number infected
Stem inoculations				
<i>F. avenaceum</i>	10	6	0	4
<i>F. oxysporum</i>	10	2	0	8
<i>F. solani</i> var. <i>eumartii</i>	10	0	0	10
<i>F. solani</i> No. 242	10	1	1	8
Summary of all <i>F. solani</i> No. 242 inoculations	195	38	23	134
Sterilized soil inoculated				
<i>F. avenaceum</i>	29	16	7	6
<i>F. oxysporum</i>	18	9	4	5
<i>F. solani</i> var. <i>eumartii</i>	20	0	0	20
<i>F. solani</i> No. 242	27	0	0	27
Summary of all <i>F. solani</i> No. 242 inoculations	101	3	13	85
Non-sterilized soil inoculated				
<i>F. avenaceum</i>	24	10	5	9
<i>F. oxysporum</i>	25	9	4	11
<i>F. solani</i> var. <i>eumartii</i>	25	0	0	25
<i>F. solani</i> No. 242	25	2	3	20

There were great differences in the percentage of infection and in the type of disease produced by these different species. Inoculations with *Fusarium solani* var. *eumartii* not only resulted in 100 per cent infection but the plants grown in inoculated soil, regardless of whether or not the soil had been previously sterilized, were all dead 4 to 5 weeks after emergence, and the stem-inoculated plants showed definite symptoms in about the same length of time. The infected plants all showed a bronzing and yellowish mottling of the leaves followed by wilting. Small, brown necrotic areas were present in the pith from the base of the stem to the top leaflet. Vascular discoloration, often with an accompanying rot, was present in the underground stems. A number of the tubers showed typical stem-end rot or vascular discoloration in the stem-inoculated plants. None of the plants grown in inoculated soil produced tubers.

Fusarium oxysporum and *F. avenaceum* did not cause so high a percentage of infection. Neither species produced a rapid wilt, all plants remained alive for 3 months with only a slight amount of yellowing. The data in table 1 regarding these 2 species are based entirely on vascular discoloration of the stem and in a very few instances of stolons and tubers.

Fusarium solani No. 242 resulted in almost as high a percentage of infection as *F. solani* var. *eumartii* but wilting did not occur. With abundant soil moisture some of the plants produced aerial tubers and the leaves sometimes became tinted with purple around the margins. Rosetting of the top occurred occasionally. These secondary symptoms would probably have been more severe with higher soil moisture and lower temperatures than were

maintained in the greenhouse (20° to 25° C.). The underground stems were always rotted, varying from a slight basal rot to a complete shattering or shredding of the stem (Fig. 1). The organism could be isolated easily from

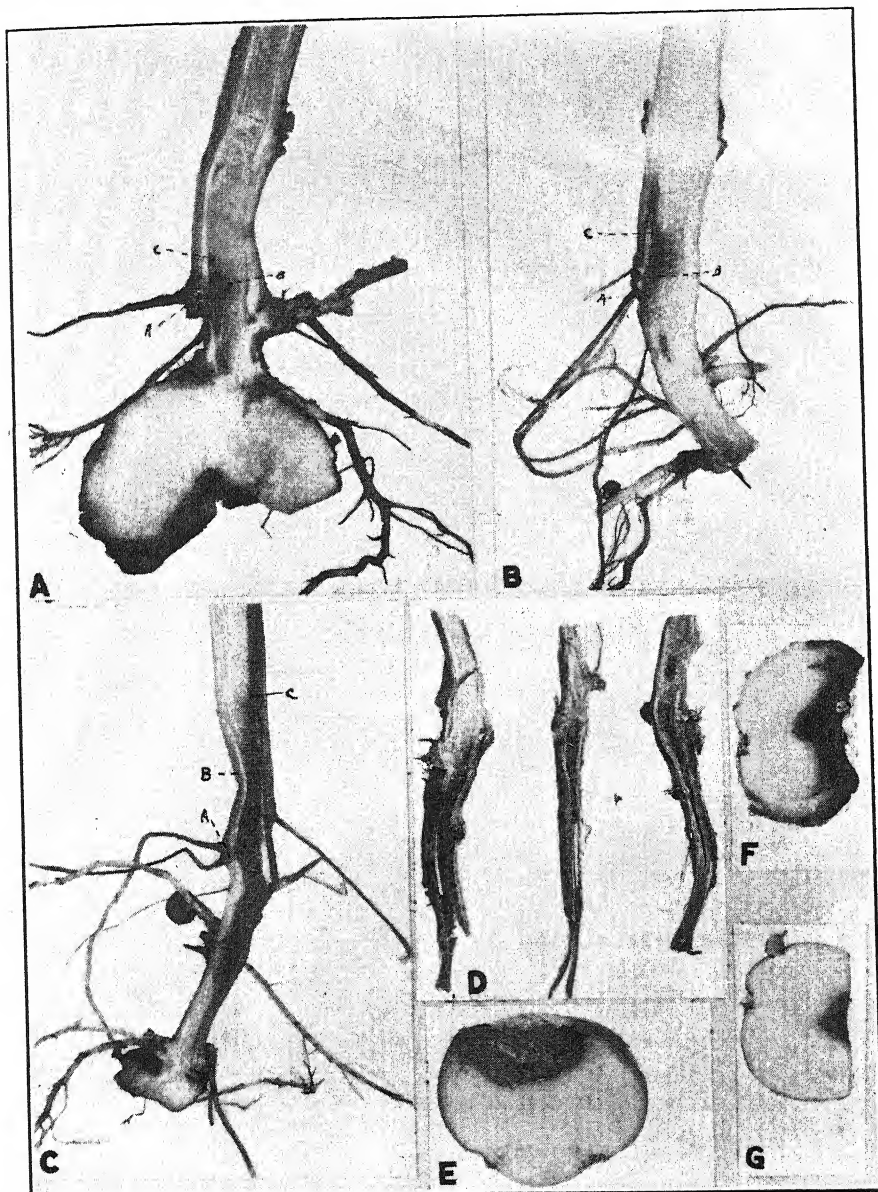


FIG. 1. A-C. Stages in the development of stem rot in Bliss Triumph plants grown in nonsterilized soil inoculated with *Fusarium solani* No. 242. Small capital letters indicate points from which the organism was isolated. D. Stems from which the original isolations were made. These are similar to the final symptoms of inoculated plants. E-F. Triumph tubers 12 and 28 days, respectively, after inoculation with *F. solani* No. 242. G. Tuber 28 days after inoculation with the squash strain of *F. solani*.

the necrotic areas of the underground stem even in the most advanced portions of the discolored pith. Evidently toxic substances causing discoloration in advance of actual penetration do not occur as with *F. solani* var. *eumartii*.

No special study was made of the mode of penetration, but the symptoms usually first appeared at the base of a root, as shown in figure 1 A, B. The absence of roots in the final stages of the disease (Fig. 1, D) indicates that this organism causes both a root and stem rot. It is possible that initial infection may occur on the roots. The emphasis placed on the stem-rot phase of the disease in this paper is due to the conspicuous and characteristic symptoms of affected stems which serve to distinguish this disease. The symptoms of the root-rot phase were indistinguishable from cortex rots of the roots due to a number of other causes.

IDENTIFICATION OF THE FUNGUS

A comparison of culture No. 242 with cultures of *Fusarium solani* showed it to be morphologically similar to this species.⁴ It has not been possible to compare it with all the varieties and forms of this species other than var. *eumartii*, from which it is quite distinct.

Fusarium solani is often considered as a common soil fungus capable of attacking weakened plant tissues or acting as a secondary invader. It also has been reported as causing root rots of a number of unrelated species of plants and the varieties and forms all typically cause root rots. As far as the author is aware, it has not previously been reported as parasitic on roots or stems of healthy potato plants.

Inoculations were made in the greenhouse with a culture of *Fusarium solani*⁵ isolated from squash, but the organism was not pathogenic on potato plants. Likewise, many other cultures, morphologically similar to *F. solani* and isolated from potato plants by the writer, have failed to produce the typical symptoms described in this paper; but some of them have been weakly pathogenic, causing limited cortex necrosis or slight vascular discoloration near the point of inoculation. Some variation has likewise been found in the virulence of different single-cell isolates of culture No. 242, but all of them were pathogenic and produced the same type of infection. A more detailed study of this strain in comparison with other strains of *F. solani* might reveal morphological differences not detected in this investigation.

TUBER ROT

The ability of this organism to cause a tuber rot as a wound parasite was tested in comparison with *Fusarium solani* var. *eumartii*. Inoculations were made by inverting a square centimeter of an agar culture on a tangential cut of a disinfected Bliss Triumph tuber and placing in a moist chamber at about 22° C. Three single-cell strains of the organism were tested, using 9

⁴ Cultures of this organism were submitted separately to Otto Reinking, C. D. Sherbakoff, and Wm. C. Snyder, and were provisionally identified by them as *Fusarium solani*.

⁵ This culture was kindly provided by Wm. C. Snyder of the University of California.

tubers for each strain. These tubers were cut open and examined at the end of 12 days (Fig. 1). The rotted tissue extended 10 to 15 mm. in depth and was light brown, soft, and contained cavities filled with hyphae. Some tubers showed a sharp line of demarcation between diseased and healthy tissue, while in others there was a softening of the tissue preceding the discoloration. In some, the browning extended further into the vascular tissue than into the pith. In contrast, *F. solani* var. *eumartii* produced a rot only about one-third as extensive and was darker brown and without cavities.

In another test including the strain of *Fusarium solani* from squash, the tubers were examined 25 days after inoculation. The squash strain was only slightly pathogenic on potato tubers, as shown in figure 1. The rot was confined to the tissue directly below the inoculum, whereas Strain No. 242 grew over the entire surface of the wound and penetrated all tissues below the wound.

TEMPERATURE RELATIONS

Preliminary tests in which the daily increase in diameter of giant Petri-dish cultures was measured indicated that the organism is favored by relatively high temperatures. The cultures were held in incubators maintained at temperatures varying from 5° to 35° at 5° C. intervals. Maximum growth occurred at 30° (84 mm. diam.) with good growth at 35° C. (34 mm.). There was only slight growth at 10° (2.5 mm.), while no growth occurred at 5° C. during the 9 days the cultures were held at these temperatures.

SUMMARY

A disease of potato is described that is characterized by a dry, shredded rot of the underground stem and the destruction of the roots resulting either in wilt or with high soil moistures, a rosetting and purpling of the leaves and the formation of aerial tubers.

The cause was found to be a strain of *Fusarium* morphologically similar to *Fusarium solani*. The disease was produced by inoculation of stems or by growing plants in inoculated soil either previously sterilized or unsterilized. The organism could be isolated from any of the discolored or rotted tissues.

Infection tests with this organism in comparison with *Fusarium oxysporum*, *F. avenaceum*, and *F. solani* var. *eumartii* showed it to be more virulent than the first two species but less so than *F. solani* var. *eumartii*. Other isolates from potato, morphologically similar to *F. solani*, and a strain from squash were not pathogenic on potato plants and only weakly so on tubers.

The organism is capable of causing a rot of potato tubers as a wound parasite, but has not been observed to infect tubers through the stolons.

It was found to be favored in pure culture by high temperatures (optimum 30° C.).

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FLOWER BLIGHT OF CAMELLIAS

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(Accepted for publication August 18, 1939)

The flower blight of Camellias (*Camellia japonica* L.), caused by a species of *Sclerotinia*, was first found in February, 1938, near Hayward, California, in a nursery where Camellias are grown under lath and in the open for cut flowers and for garden plants. Apothecia were found in abundance under the plants grown in the lath house, whereas neither apothecia nor sclerotia were found under those grown in the open where field cultivation was practiced and no mulches used. Apparently, the flowers grown in the open became infected by wind-borne spores produced in the adjacent lath house.

Only the floral parts are affected, and all of the more than 50 varieties of Camellias grown in this nursery appeared to be equally susceptible. The disease is highly specific to this one host, as shown by the fact that no infections were found on flowers of Rhododendrons, Azaleas, Magnolias, Gardenias, Paeonies and many other flowering shrubs, though they were grown under the same lath, intermingled with Camellias. We have examined several places in the State where Camellias are grown and made inquiries at others, but obtained no evidence that the disease had been observed in any of them. The only suggestion of a possible source of this new disease may be seen in the fact that Camellias are native to the Orient and are still being freely imported from there.

The occurrence of the disease coincides with the flowering period of Camellias, roughly from February to May, which is also the season of rather frequent rains. During that period the losses vary from a relatively small percentage in dry weather to 100 per cent of all open flowers for several days following rains. Under such humid conditions it is unsafe to cut and ship even the flowers that appear to be unaffected, as they become badly spotted in transit and storage.

SYMPTOMS

Infection of the individual flower may take place soon after the tips of petals are visible in the opening bud or at any time thereafter. Few to many small, irregular, brownish specks appear on the petals of expanding flowers (Fig. 1, A, B). Under favorable conditions of temperature and moisture these specks rapidly enlarge and unite to form large spots (Fig. 1, B) which soon involve the entire petal and eventually the whole flower, which becomes uniformly dull brown and drops from the plant. Even one or two infections may suffice to render the flower unsalable. When infection begins near the base of the petals the entire center of the flower may be killed, while the tips of the petals retain their normal form and color. There is no rapid disintegration of invaded tissues, hence infected flowers retain their shape and firmness for many days after they have turned completely brown and fallen

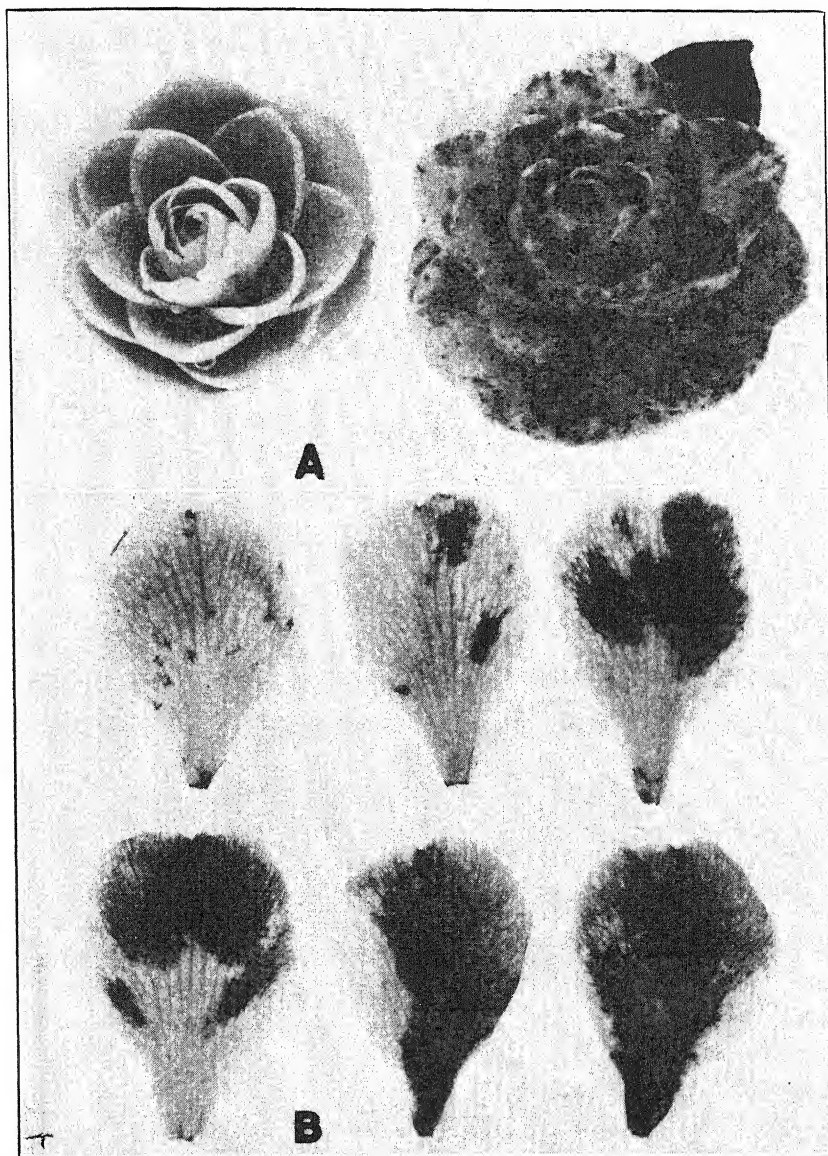


FIG. 1. A. Camellia flower 24 hours after being inoculated with ascospores of *Sclerotinia camelliae* (control on left). B. Individual petals from naturally infected flowers showing early and late stages of the disease.

to the ground. While the flowers are resting on the wet earth, microconidia are often produced on the petals in shiny, black streaks or masses, giving the flowers the appearance of being affected with a wet rot. After the flower is completely blighted the causal fungus continues to develop within the basal parts of the petals, but soon grows beyond the petal limits (Fig. 2, D) to form hard, dark-brown to black sclerotia that frequently unite at the base

to form a laminated compound structure simulating the imbricate petal arrangement in the flower (Fig. 2, A).

LIFE HISTORY OF THE FUNGUS

The sclerotia lie dormant on the ground or covered with soil or mulching materials under the bushes during the summer and early part of the winter. As the period of *Camellia* bloom in early spring approaches, some of the sclerotia become active, while others remain dormant for another year or possibly longer. After a period of wet weather with rising temperature, they then begin growth and produce from one to several apothecia each. Apothecial formation is apparently greatly stimulated by spring applications to the soil of top-dressing materials, such as barnyard manure, peat, etc. In cases where the sclerotia are near the surface, the apothecia may be nearly sessile or, where deeply buried, the stipes may be up to 40 mm. in length (Fig. 2, B). The saucer-like discs of the apothecia vary from 5 to 20 mm. in diameter. The ascospores are discharged forcibly into the air and are carried by wind currents to the flowers, where they germinate, invade the petal tissues, and eventually produce sclerotia and microconidia, thus completing the life cycle. Flowers at the top of a 15-ft. bush appear to become infected as readily as those produced near the ground. The microconidia have not been observed to germinate and no secondary conidia are formed.

BEHAVIOR ON ARTIFICIAL MEDIA

On potato dextrose agar the fungus first forms a very close, felty, cream colored mat which begins to turn darker in about 10 days and becomes jet-black and shiny in about 25 days, the change in color being due to the presence of microconidia, which are produced in abundance over the entire mat. Occasionally small, flat, black sclerotia are formed that rarely attain a size of more than 2×5 mm. On sterilized whole wheat, well-rounded sclerotia up to 7 mm. in diameter have been produced. The fungus produces microspores sooner and more abundantly at room temperature, about 24° C., but mycelial growth and sclerotium production is much more rapid at 15 to 18° C.

INOCULATION

Six detached healthy *Camellia* flowers were sprayed with water and suspended for 1 hour over mature, spore-discharging apothecia. Six others were sprayed with a water suspension of ascospores. After inoculation the flowers, together with noninoculated controls, were placed in moist chambers. All the inoculated flowers showed typical spotting after 24 hours (Fig. 1, A) and became completely browned in 48 hours, with sclerotial formation well started. The controls remained unspotted.

A careful search of the literature failed to reveal any Discomycete associated with *Camellias* or with other members of the family *Theaceae*. In view of this, and on the basis of certain distinct morphological features, to-

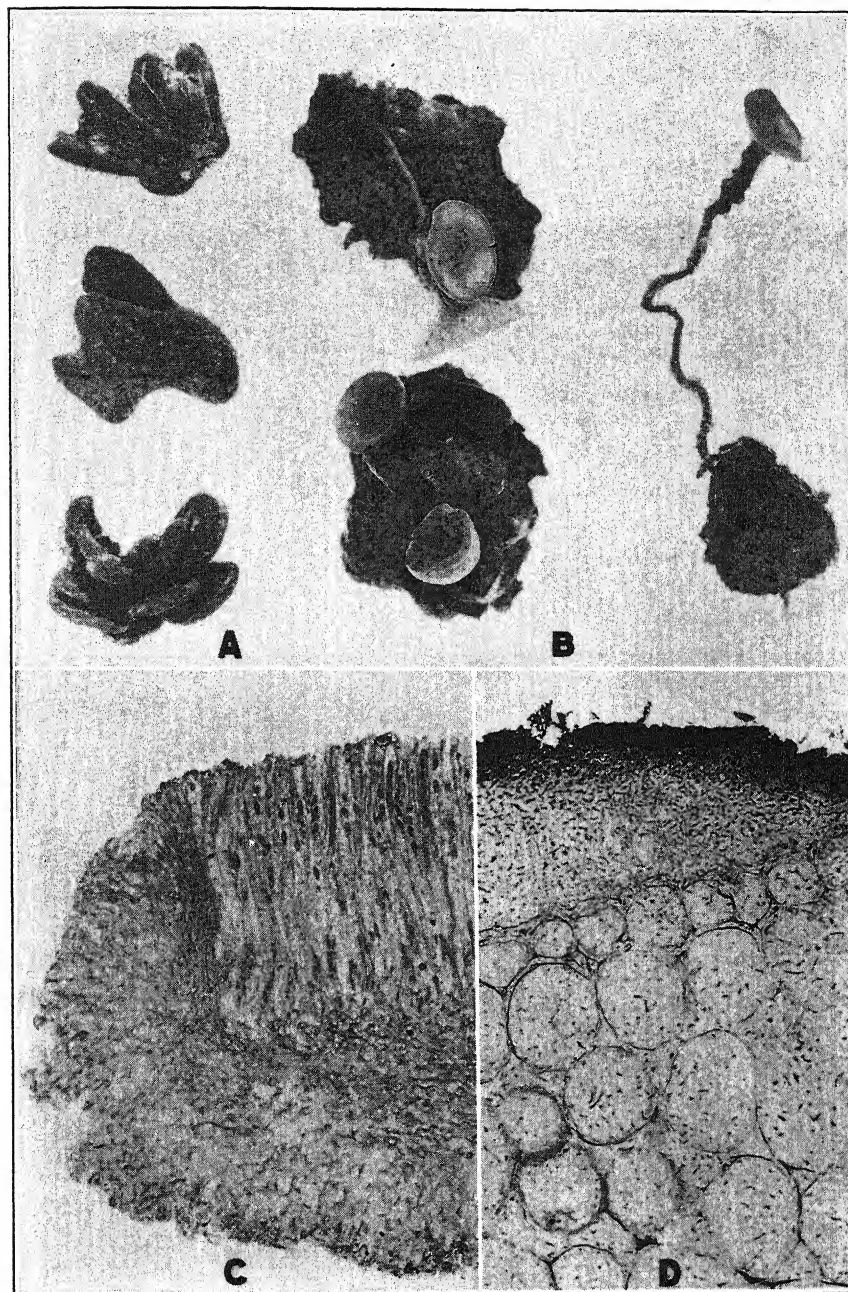


FIG. 2. A. Typical petaloid sclerotia. B. Short-stipe and long-stipe apothecia. C. Part of cross section of apothecium. $\times 380$. D. Part of cross section of sclerotium. $\times 185$.

gether with the host specificity of the pathogen, we conclude that it is new to science and propose for it the name *Sclerotinia camelliae*, sp. nov.

TECHNICAL DESCRIPTION OF THE PATHOGEN

Sclerotinia camelliae, sp. nov. Apothecia singly or in groups, buff-olive becoming darker with age, scantily pubescent; disc cyathiform becoming discoid, 5–20 mm. in diameter; stipe 3 to 40 mm. long, 2–3 mm. in diameter below disc tapering to 0.5–1.0 mm. at base. Asci cylindrical 4.3–5.8 \times 100–125 μ . Ascospores 8, uniseriate, ellipsoid, continuous, hyaline 2.5–3.5 \times 5.3–7.0 μ . Paraphyses filiform, septate 1.2–2.5 \times 110–130 μ , tips slightly swollen. Sclerotia dark-brown to black, usually compound, impregnating and surrounding the petal tissues; very variable in outline, up to 12 \times 30 mm. in size; usually laminated to simulate the imbricate petal arrangement of flowers. Conidia none. Microconidia globose to pyriform 2.5–3.5 μ , catenate, hyaline under high magnification, jet-black in mass, produced in a sporodochium made up of numerous clusters of conidiophores that end in tapering elongate terminal cells on which the long chains of microconidia are produced.

CONTROL

Our observation during the past 2 seasons that no apothecia were found under plants grown without mulching in the open would suggest that there is little danger of the disease becoming established in parks or private gardens. In view of the facts that ascospores alone are able to cause infection and that sclerotia are formed in the flowers only, it would seem a relatively simple matter to control the disease and eradicate the pathogen by gathering and destroying all fallen flowers. This has been undertaken by the nursery involved. This would have to be done for at least 2 consecutive seasons, since it is known that the sclerotia may remain alive in the soil for at least 2 years and probably longer.

SUMMARY

A new disease affecting the flowers of *Camellia japonica* L. is described. Early symptoms on the petals appear shortly after late winter and spring rains as small brown specks, which soon enlarge, coalesce, and cause the whole flower to turn brown and fall. Sclerotia, formed within the flower, rest on or in the ground for one or more years after which they produce apothecia. The ascospores are wind-borne, and there are no viable conidia. The pathogen, described as new, is named *Sclerotinia camelliae*, sp. nov. Destruction of all fallen flowers for several consecutive seasons is suggested for control.

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SOME EFFECTS OF STRAINS OF CUCUMBER VIRUS 1 IN LILY AND TULIP

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(Accepted for publication August 8, 1939)

On December 20, 1938, healthy seedlings of the Easter lily (*Lilium longiflorum* Thunb.) were inoculated with 5 strains of Cucumber Virus 1, including one isolated from Easter lily, each taken from Turkish tobacco, and a lily virus of the tulip group taken from Easter lily seedlings. After 34 days all plants inoculated with the cucumber-virus strains remained essentially symptomless, but those inoculated with the tulip virus expressed characteristic strong mottling from about 14 days onward (Fig. 1, A). On subinoculation from each set of 5 lily plants to Turkish tobacco, all 5 cucumber strains were recovered (Fig. 1, B) but the tulip virus was not. In other trials the common lily strain of cucumber mosaic has been passed through both seedling Easter lilies and through *L. formosanum* Stapf. many times, and through *L. speciosum* Thunb. once, without inducing well-defined symptoms. From about May 15 onward, when the greenhouse temperatures rise above control in this latitude, a mild yellow mottling has appeared in young leaves of *L. formosanum* inoculated with lily strains of cucumber mosaic some days or months previously. No well-defined effects in inoculated Easter lily seedlings or in *L. speciosum* accompanied this seasonal rise in temperature.

Flecking, roughly comparable to the necrotic-fleck type seen in commercial Easter lilies, has been produced by inoculating a lily strain of cucumber mosaic into commercial Easter lilies, shown by previous subinoculation to carry McWhorter's (3) "latent virus of lily" (Fig. 1, C). The most plausible explanation of the discrepancy between our results and Price's (5) report of fleck symptoms induced by cucumber-virus strains alone is that some of Price's "selected cage-grown stock" carried the latent virus reported by McWhorter, *i.e.*, a virus of the tulip group, typically latent in *Lilium longiflorum*. Furthermore, Wellman's (6) gray mottle and flecking in Easter lilies inoculated with the celery strain indicates that the inoculated Florida stocks already carried a virus of the tulip group.

Cross inoculations of viruses from tulip to cucumber and tobacco, and of the cucumber-virus strains from cucumber to tulip were negative in our trials until this year (2). However, on March 30 and May 2, 1939, we twice successfully subinoculated to tobacco the celery strain and a lily strain of Cucumber Virus 1 from Clara Butt tulips inoculated with these viruses on April 16, 1938. Parallel subinoculations to tobacco from Clara Butt tulips inoculated with viruses of the tulip group from tulip and lily were negative. Type material of McWhorter's (4) tulip viruses 1 and 2 readily produced symptoms in *Lilium formosanum*, but no infection in Turkish tobacco.

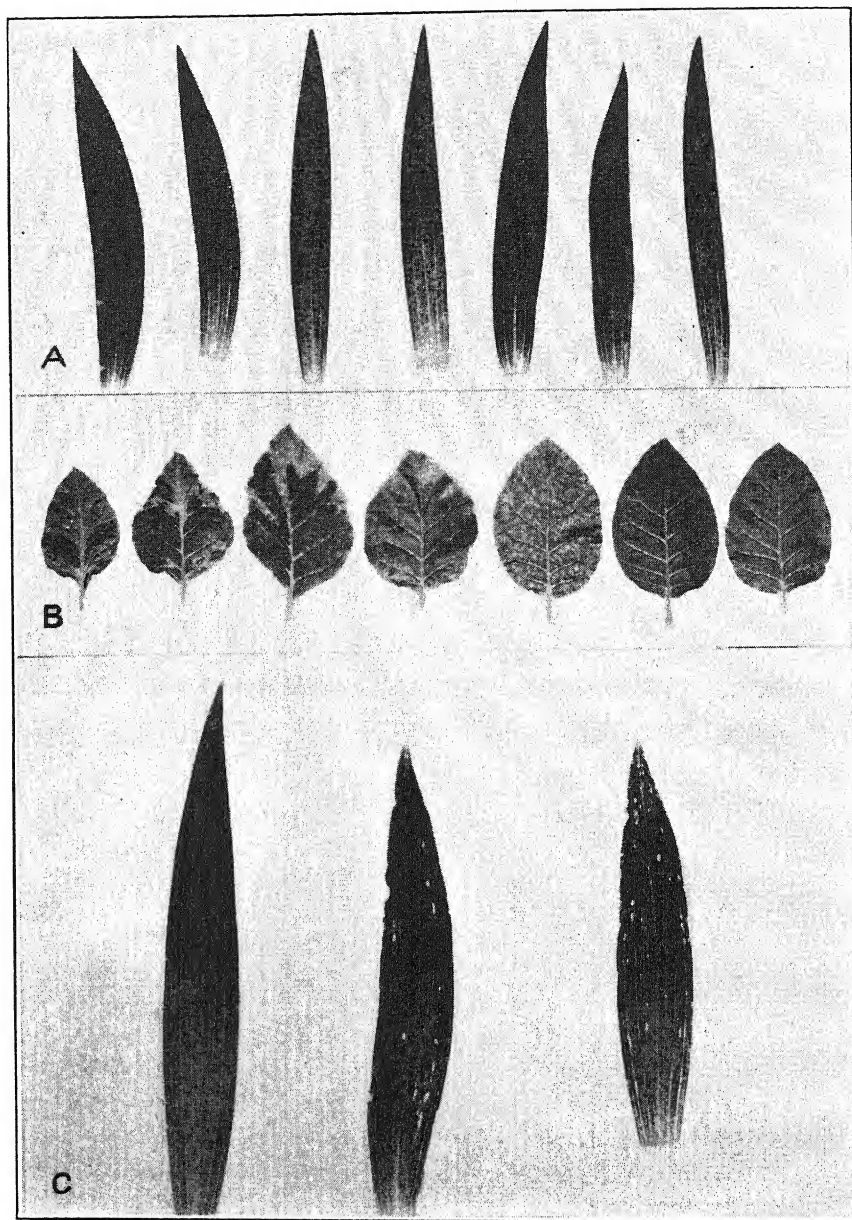


FIG. 1. A. Representative leaves from each of seven sets of Easter lily seedlings inoculated with (left to right) Doolittle's strain, a second isolate similar to the first, Price's strain, celery strain, and a lily strain of Cucumber Virus 1, the strong mottle virus (tulip type) from Easter lily, and uninoculated control. B. Representative leaves from sub-inoculations to Turkish tobacco from the corresponding Easter lily inoculations shown above. C. Representative leaves from three sets of an Easter lily clon carrying McWhorter's latent virus of lily (tulip virus). Left to right: uninoculated control, symptomless; fleck symptoms following inoculation with a lily strain of Cucumber Virus 1; fleck symptoms following inoculation from typical flecked Easter lily.

Clara Butt tulips, from which the celery and lily strains of cucumber virus were reisolated, showed longitudinal gray streaks in the leaves at about the time the blooms began to show color. The flowers were broken (Fig. 2, B) with a dull break, the margins of the stripes being less sharply defined than in tulip mosaic (Fig. 2, C). The outer perianth parts usually showed

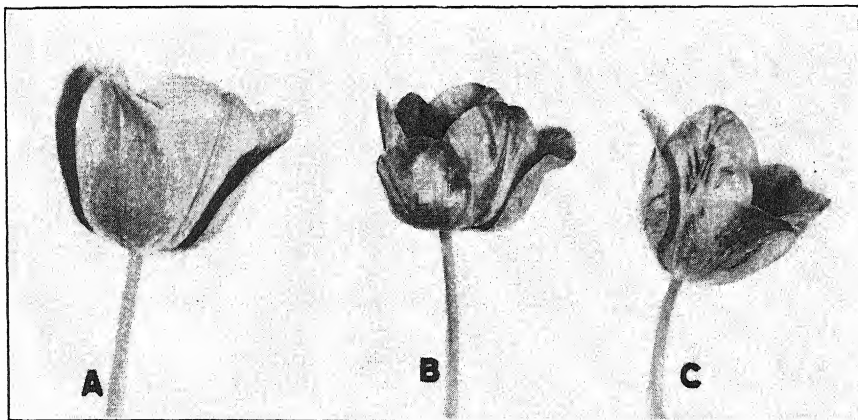


FIG. 2. Two types of flower breaks in Clara Butt tulips. Left to right: A. Healthy. B. Broken from inoculation April 16, 1938, with the common lily strain of Cucumber Virus 1. C. Natural infection with tulip mosaic.

a characteristic blemish (Fig. 2, B) and were shorter than normal. The blemish was gray at the base, sometimes greenish at the summit. Subinoculation from plants showing this atypical break to Turkish tobacco were uniformly successful in recovering the celery or the introduced lily strain, while parallel subinoculations to *Lilium formosanum* gave no evidence of the presence of tulip virus. The symptoms illustrated are, therefore, those of cucumber virus (lily strain) in tulip. The type has not been observed by the writers in previous experience with tulips.

The susceptibility of tulips to other strains of Cucumber Virus 1 was expected after Ainsworth (1) reported isolation of a strain of this group from tulip. Our results show that a cucumber virus (celery strain or lily strain) may be introduced into tulips and induce breaks in the blooms, but that cucumber strains are not associated with the usual breaking of tulips. In Easter lilies, strains of cucumber mosaic are recoverable from plants thus far sampled showing any of the distinctly injurious symptom types, but the cucumber strains alone are, with one known exception, not markedly damaging to Easter lilies. It is increasingly evident that lilies are common hosts for members of the tulip and the cucumber 1 groups of viruses, but there is no evidence from our work, or from any yet published by others, to indicate that these groups are closely allied.

We have not found cucumber virus strains occurring naturally in tulips nor yet alone in commercial Easter lilies. In our tests, when such strains are experimentally introduced into seedling Easter lilies, they have not induced

symptoms with the exception of one virulent strain, associated with yellow top symptoms rather than fleck symptoms. McWhorter's (3) "latent virus of lily" is very commonly present in symptomless Easter lilies, excepting suitably protected seedlings. It is reasonable to assume that the tulip type latent is so prevalent in commercial Easter lily stocks that infection with a cucumber strain commonly produces a complex with recognizable symptoms.

It is of interest that cucumber mosaic strains, experimentally introduced into tulips, produced no recognized effects in the current season, but induced flower breaks in the following year, as do the tulip viruses. On the other hand, the tulip viruses from lily and tulip, including those received from McWhorter as type material, induce symptoms in *Lilium formosanum* in 2 weeks. The long incubation period of the classical tulip breaking is, therefore, evidently a peculiarity of the tulip rather than of the viruses involved.

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DEVELOPMENT OF SCAB ON STORED APPLES, 1938-1939

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(Accepted for publication September 21, 1939)

In February, 1939, the writer observed several lots of apples from Pennsylvania, New Jersey, Massachusetts, and the Hudson Valley of New York on the New York City market bearing many small, black, scab lesions typical of those that develop in storage. The lesions on Stayman Winesap, Baldwin, and Stark varieties were mostly from $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter and were jet black. Lesions on Rome Beauty apples were smaller, being generally less than $\frac{1}{16}$ inch in diameter, and were dark brown. A few of the lesions, particularly those on riper fruits, were larger, caused no roughening of the cuticle of the apple, and resembled "ink spots." The cuticle on most of the lesions, however, was roughened and bore low fungus growth. Typical lesions, as they appeared on apples of these 4 varieties, are shown in figure 1. The Stayman Winesap apple in the photograph bore a total of 553 lesions.

Some of the scabbed lots were traced to their points of origin in the eastern part of Pennsylvania, where the writer examined additional lots of apples in storage and interviewed growers and cold-storage warehouse managers concerning treatment of the fruit. Many of the lots of Stayman Winesap and Rome Beauty showed from 80 to 90 per cent of the fruits in-

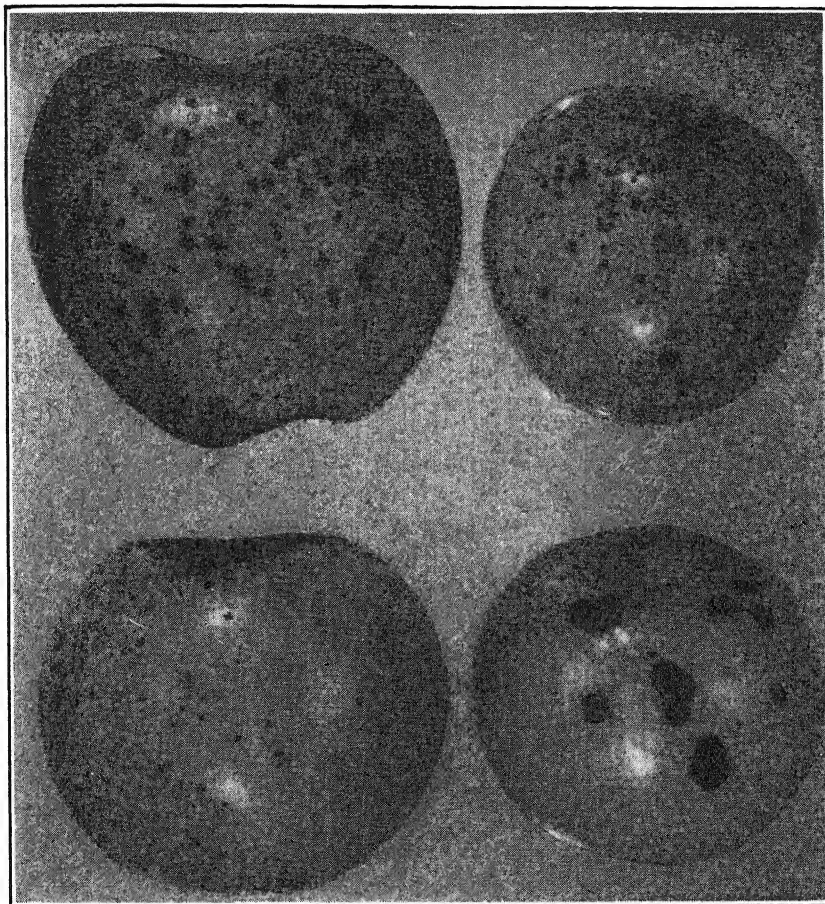


FIG. 1. Scab on apples held in cold storage for 4 months. Apples on left from Pennsylvania, Stayman Winesap above, Rome Beauty below; apples on right from Hudson Valley of New York, Baldwin above, Stark below.

ected. Apples from 1 orchard stored in 3 different cold-storage warehouses were equally affected, indicating that any slight differences in storage conditions that the fruit may have been subjected to had little effect on scab development. McIntosh and Delicious apples, picked from the same orchard and stored in the same cold storage as that of the heavily infected fruits of Rome Beauty and Stayman Winesap varieties, were found to have remained scab-free. By reference to the records kept by the grower, it was learned that the first named varieties were picked and stored during the first 3 weeks of September, whereas the Rome Beauty and Stayman Winesap varieties were picked during the first 3 weeks of October.

The growers' records showed that the orchards from which the scabbed Pennsylvania apples came received the final spray application during the first week of July. Foliage scab was apparently so well controlled that a later spray was not considered necessary. It was stated that at picking

time foliage scab was slight and very few fruits bore visible scab lesions. No new lesions were seen on the stored fruit by those who inspected it until January, when very small spots were seen on an occasional fruit.

Information concerning the prevalence of scab on stored fruit grown in other sections of the Northeast was obtained through correspondence with growers and inspectors of the Agricultural Marketing Service of the U. S. Department of Agriculture. The McIntosh variety in the Hudson Valley, which was picked mostly during the first 3 weeks of September, remained nearly scab-free, whereas the Baldwin, Stark, and Rome Beauty varieties, picked in October, became in many cases severely spotted during storage. Reports from New Jersey and New England indicate that only those varieties picked in October became heavily infected. Orchardists in Connecticut stated that the windfalls picked up and stored immediately after the hurricane of September, though not held so long as the fruit picked afterward, showed little scab development, whereas fruit picked later showed heavy infection. Growers in Massachusetts reported that heavy storage infection occurred on Baldwin and other varieties of apples picked in October. Contrary to the general situation throughout the Northeast, little scab development on stored apples was reported from western New York.

Inoculation experiments reported earlier by the writer¹ showed that apple fruits could be successfully inoculated at any stage of their development on the tree provided they were subjected to increasingly longer periods of wetness as they enlarged. Apples nearing maturity and mature fruits picked from the tree were successfully inoculated when kept moist at orchard temperature for 7 days. If the apples were picked and stored immediately after the 7-day period, the resulting infection did not appear until the normal storage life of the fruit was passed; but, if they were allowed to remain on the tree for a week or longer, the latent period of infection was shortened to from 2 to 4 months.

In an attempt to correlate this outbreak of scab with weather conditions in late summer, weather reports were obtained from U. S. Weather Bureau Stations in representative cities in the Northeast. Throughout this section rains occurred on several successive days in early August but, at most stations, high percentages of sunshine were recorded on the days of the rains. Foliage and fruit apparently did not remain wet long enough during this period to allow serious infections, for resulting lesions should have appeared on the fruit by late September or early October.

Only light rains on occasional days were reported at any of the stations during the early part of September. But starting September 12 and lasting through the hurricane, 9 days later, light to heavy rains occurred generally throughout the East. At New York City some rain fell daily during that period, and at other nearby stations only 1 or 2 days were entirely rain-free. Table 1 shows daily measure of precipitation at the 7 stations from which records were obtained. With the exception of those for Rochester, N. Y.,

¹ Bratley, C. O. Incidence and development of apple scab on fruit during late summer and while in storage. U. S. Dept. Agr. Tech. Bull. 563. 45 p. 1937.

TABLE 1.—Precipitation recorded from September 11 to 22, 1938, by the U. S. Weather Bureau at 7 stations in the Northeast

Station	Precipitation in inches per day in September											
	11	12	13	14	15	16	17	18	19	20	21	22
Reading, Pa.	T	T	.05	.23	.01	0	.31	.01	.60	1.77	1.12	0
Trenton, N. J.	0	0	.02	T	.54	T	.34	.38	2.46	2.27	2.67	0
New York, N. Y.	0	T	.13	T	.66	T	.12	.39	1.80	1.73	3.71	0
Albany, N. Y.	0	.45	.24	.23	.26	0	.25	.01	1.78	1.25	3.25	0
Rochester, N. Y.	0	.26	.04	1.51	.03	.08	T	0	.17	0	.65	1.33
Hartford, Conn.	0	.07	.27	T	.51	0	.60	1.45	1.80	6.10	3.22	0
Boston, Mass.	0	.27	.65	0	.36	0	.56	1.19	.67	1.50	.10	0

the records show moderate rainfall from September 12 to 15, then one day, the 16th, with little or no rain followed by very heavy rains daily from the 17th to and including the 21st. During the latter period of 5 days little or no sunshine was reported and relative humidity averaged 90 per cent or above most of the time. These 5 days, preceded as they were by an almost equal period of intermittent rains, undoubtedly afforded optimum conditions for inoculation of apple fruits. This is borne out by the fact noted above that only those apples picked after September 21 showed extensive development of scab in storage.

As may be seen by reference to table 1, Rochester, N. Y., did not receive the heavy, almost continuous rains from September 17 to 21. There was some rain daily at this station during the period from September 12 to 17, but with the exception of the 12th, 14th, and 15th, the daily percentages of sunshine reported were quite high and the percentages of relative humidity fairly low. It is, therefore, interesting to note that no more than the usual amount of scab development in storage was reported on fruit from western New York of which Rochester is the center.

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THE SNOW MOLDS OF GRAINS AND GRASSES CAUSED BY *TYPHULA ITOANA* AND *TYPHULA IDAHOENSIS*

RUTH E. REMSBERG

(Accepted for publication Sept. 20, 1939)

INTRODUCTION

A great deal of confusion exists in phytopathological literature concerning the identity of the organisms causing the snow molds of grains and grasses. The sclerotial fungi that have been found associated with these diseases have been referred at various times to *Sclerotium rhizodes* Auerswald¹ (2), *Scl. fulvum* Fries (11), *Typhula graminum* Karst. (9, 10) and *T. itoana* Imai (4). This confusion has come about partly because of the macroscopic resemblance of the sclerotia of the above-named fungi as described by early mycologists, and partly because of the inability to collect or otherwise obtain fertile sporophores, which are essential for taxonomic classification. A recent study made by the writer (8) has cleared up this situation, and the species of *Typhula* associated with these diseases have been identified.

REVIEW OF LITERATURE

The snow molds have been of considerable economic importance in the United States, as well as in Europe and Japan. They are associated with cold weather and snow, and are most frequently found where the snow is deep or drifted and, consequently, delayed in melting in the spring. When

¹ According to H. H. Whetzel, *Sclerotium rhizodes* Auers. is a *Rhizoctonia*. Unpublished data.

the snow melts, the plants are found covered with a moldy, white fungous growth, and the tissue is filled with small reddish brown or dark brown sclerotia. These diseases have caused considerable damage in Idaho (7) and Montana (11), and *Typhula itoana* is becoming of increasing importance on turf and lawn grasses in the eastern United States. For several years *T. itoana* has been the cause for much concern in Japan (5, 9) and frequently has been reported from northern Europe (6, 10) and the Scandinavian countries (1), usually under the name *T. graminum* Karst.

TAXONOMIC POSITION OF THE FUNGI

Since it is possible to fruit these fungi abundantly and at will in either diffuse natural daylight or under ultra-violet irradiation (8), it is an easy matter to identify them. The fungus most commonly found is *Typhula itoana* (3), which produces tawny to hazel-brown sclerotia from which develop rose-colored sporophores, 8–25 mm. tall. An examination and comparison of cultures obtained in the United States with those from Japan² and Europe³ show that the same organism, *T. itoana*, is the cause of a snow mold in these three countries. Ekstrand (1) has called attention also to the fact that the most common species causing this same disease in Sweden is *T. itoana*.

A second species of *Typhula* has been found associated with snow mold in Idaho and Montana. This one produces small chestnut brown sclerotia from which develop fawn to wood-brown sporophores, 5–10 mm. tall. This species has been described as *Typhula idahoensis* (8), and causes a disease of the same type as that caused by *T. itoana*.

Ekstrand (1) mentions in addition to *Typhula itoana*, a small brownish black sclerotium, which is frequently isolated from diseased cereals and grasses in northern Sweden. He has given the name *T. borealis* to the sclerotial stage of the fungus, but does not describe the sporophores. From his short description of the sclerotia it seems possible that he is dealing with a species similar to, if not identical with, *T. idahoensis*.

It is necessary to study the sclerotial morphology of species of *Typhula* before a proper identification can be made. Sclerotia of different species often resemble each other very closely macroscopically, but have an entirely different arrangement of rind and medulla, and differ also in the characters of the sporophores they produce. The morphological construction of sclerotia of *T. itoana* is identical with that of *Sclerotium fulvum* Fries⁴; that is, the rind is a homogeneous gelatinous layer and the medulla paraplectenchymatous (8). It is safe to assume that these two organisms are the same. On the other hand, a critical examination of the type specimen of *T. graminum* Karst.⁵ shows the sclerotia to be of a different morphological type.

² Obtained through the courtesy of H. Tasugi, Tokyo, Japan.

³ Obtained through the courtesy of A. Volk, Königsberg, Germany.

⁴ Roumeguère, *Fungi gallici exsiccati*, 1400, *Sclerotium fulvum* Fr., in the Herbarium, Dept. of Plant Pathology, Cornell University, Ithaca, N. Y.

⁵ Loaned for examination through the courtesy of Dr. Harald Lindberg, Helsingfors, Finland.

The medulla is prosoplectenchymatous with a layer of enlarged thin-walled cells adjacent to the homogeneous gelatinous rind. The macroscopic similarity of sclerotia of *Scl. fulvum*, *T. graminum* and *T. itoana* has doubtless led many investigators to assign all the organisms causing the snow molds to *T. graminum*. *T. graminum*, however, produces white sporophores, while *T. itoana* produces rose-colored ones. There is a possibility that *T. graminum* also causes a snow mold, but its isolation and pathogenicity have never been conclusively demonstrated by any investigator. It is interesting to note that the sporophores of *T. graminum* Karst. do not arise from the sclerotia of *Scl. fulvum* Fries, as Karsten originally assumed.

SUMMARY

Two species of *Typhula* are definitely associated with snow molds of cereals and grasses. The one most commonly found in the United States, Europe and Japan is *T. itoana* Imai, frequently described under the name of *T. graminum* Karsten or *Sclerotium fulvum* Fries. The second species, often collected in western United States, is *T. idahoensis* Remsberg, and is probably the organism described by Ekstrand as *T. borealis* in Sweden. There is a possibility that a third species, *T. graminum* Karsten, may be associated with the snow molds, but it has not yet been demonstrated.

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PHYTOPATHOLOGICAL NOTES

Potato Seed-piece Rot Caused by Fusarium oxysporum.—In 1934, a potato seed-piece rot was observed at Hastings, Florida, in many fields within 2 to 3 weeks after potatoes were planted. The disease caused stand reductions varying from 10 to 70 per cent in 400 acres, planted in December, 1933 (Fig. 1, A). It has reappeared every year since 1934, but has caused little trouble, except in 1936, when it destroyed 10 per cent or more of the seed in most of the potato fields in the Hastings section.

Gratz¹ reported a *Fusarium* seed-piece rot at Hastings, Fla., in 1924 and 1925, but he did not identify the species of *Fusarium* concerned. In 1919, MacMillan² reported that infection of potatoes by *Fusarium oxysporum* Schlect from the soil through the seed piece occurred in irrigated land in potato-growing sections of Colorado. MacMillan described the malady in detail and suggested that its severity in some soils was due to growing successive crops of potatoes on the land and to the presence of soil temperatures especially favorable for the disease.

The *Fusarium* causing seed-piece rot can be seen on the cut surface and occasionally on the cortex of decaying seed pieces 2 to 3 weeks after planting (Fig. 1, C and D). The rot starts at the cut surface, and the tissues invaded become brown (Fig. 1, B) but remain firm until secondary decay organisms enter, after which the pieces become soft and mushy. If decay is rapid, the seed pieces either fail to germinate or produce weak sprouts that may rot off before they can emerge from the soil. If the affected seed pieces decay slowly, they may produce plants bearing marketable tubers or plants that produce only a few small tubers or none when the fungus destroys the seed, invades the stems and roots, and finally causes the plant to wilt and die prematurely. Tubers of plants with affected seed pieces do not rot in the field.

Hard potato-dextrose agar of pH 5.6 was colored vinaceous purple by the fungus isolated from decaying seed pieces. The macroconidia were typically 3-septate, $4.5\ \mu \times 40.3\ \mu$, borne on branched conidiophores. Macroconidia formed in sporodochia and chlamydospores developed in aged cultures. The characteristics of the organism (Fig. 2) agree with those given by Sherbakoff³ for *Fusarium oxysporum*.

In fields where the disease was severe in 1934, it was noted that seed became infected and rotted in one row where no fertilizer had been used, in rows fertilized 2 weeks prior to planting, and in rows where the seed and fertilizer had been placed in the soil at one operation.

Numerous inoculation experiments proved that *Fusarium oxysporum* isolated from decaying seed pieces was pathogenic. In one series of experiments, pure cultures of the fungus growing on small squares of potato-dex-

¹ Gratz, L. O. Irish potato disease investigations, 1924-25. Fla. Agr. Exp. Stat. Bull. 176. 1925.

² MacMillan, H. G. *Fusarium* blight of potatoes under irrigation. Jour. Agr. Res. [U. S.] 16: 279-303. 1919.

³ Sherbakoff, C. D. *Fusaria* of potatoes. Cornell Agr. Exp. Stat. Mem. 6. 1915.

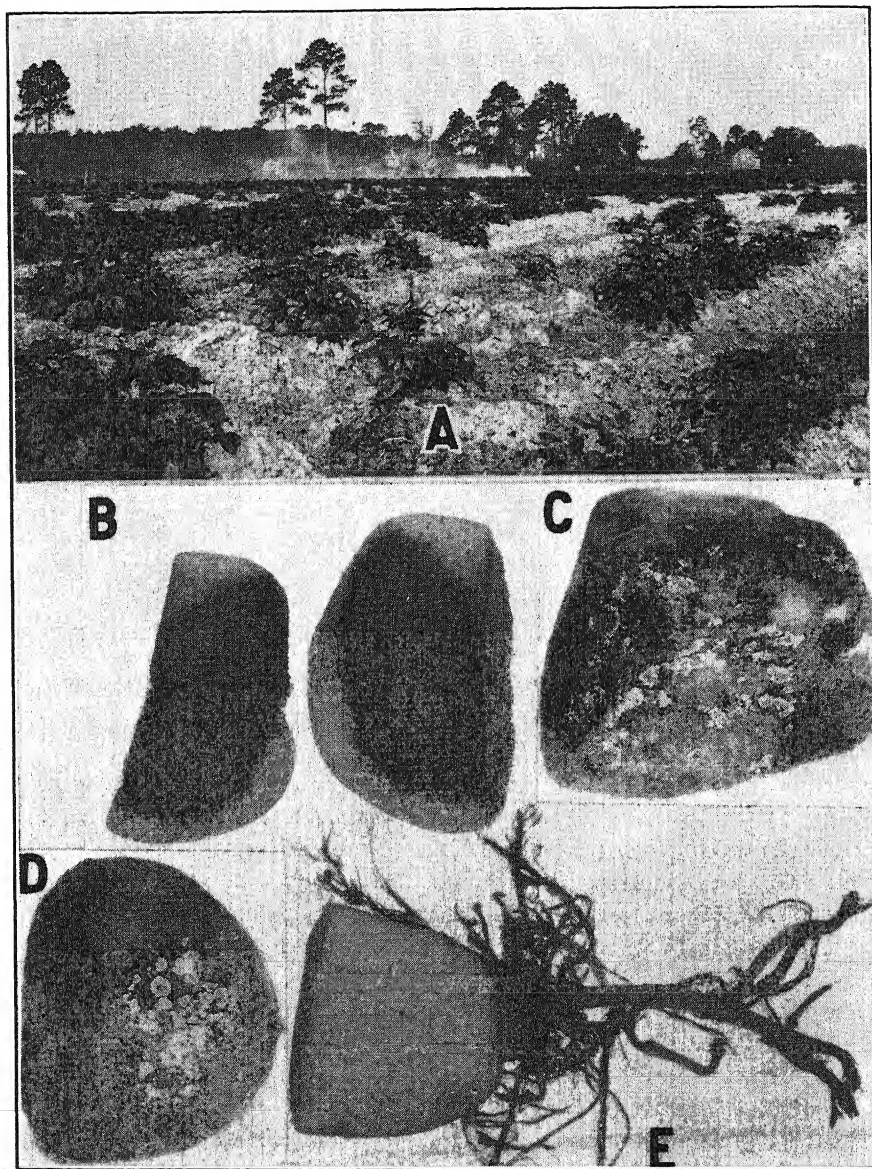


FIG. 1. *Fusarium* seed-piece rot of potato. A. Section of a field in which 65 per cent of the seed pieces were destroyed by *F. oxysporum* prior to germination. B-D. Naturally infected seed pieces: Seed piece, which did not germinate, sectioned to show discoloration caused by *F. oxysporum* (B); *F. oxysporum* growing on cut surfaces (C) and on cortex (D). E. Section of sprouted healthy seed piece.

trose agar were placed on the cut surfaces of seed pieces which had been cut one day previously and those that had been cut only a few minutes before being inoculated. The inoculated pieces were planted in sterilized soil, together with noninoculated pieces from the same seed tubers. When re-

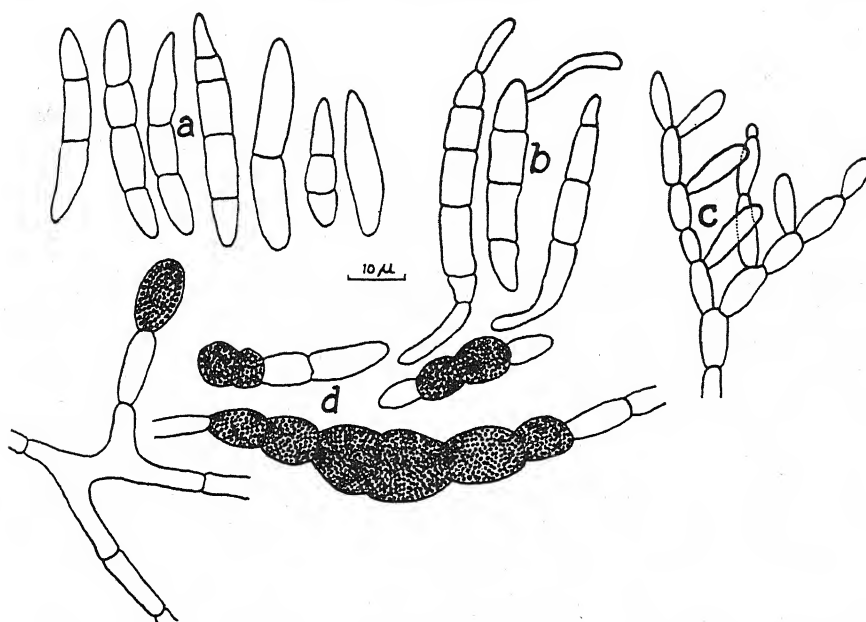


FIG. 2. Camera-lucida drawings of *Fusarium oxysporum* showing (a) types of macroconidia, (b) germinating macroconidia, (c) conidiophore and (d) chlamydospores in mycelium and macroconidia.

moved from the soil 28 days later, all inoculated pieces were partly or totally decayed and either had not germinated or had produced only weak sprouts, while the pieces not inoculated were sound and had produced strong sprouts.

In another series of experiments, 25 inoculated and 25 noninoculated seed pieces from the same seed tubers were planted in a field in rows in which one ton of fertilizer per acre had been distributed 2 weeks before planting. When examined 28 days later, all inoculated pieces were partly or totally decayed and 14 of the noninoculated pieces were also rotting. *Fusarium oxysporum* was isolated from the inoculated and noninoculated pieces that were rotting.—A. H. EDDINS, Hastings Laboratory, Florida Agricultural Experiment Station, Hastings, Florida.

Lightning Injury of Black Locust Seedlings.—An occurrence of lightning injury of first-year black locust (*Robinia pseudoacacia* L.) seedlings, which had just emerged, was observed during July, 1936, in a forest nursery near New Brunswick, New Jersey. According to information from the nursery personnel, the lightning entered the field of seedlings from a power-line tower. The damaged spot was approximately 50 by 100 feet, and it extended from one side of the tower (Fig. 1, A). Most of the plants in the central part of the spot were killed immediately. Those in the outer part were severely injured but not killed, as shown by the various degrees of the dwarfing that appeared later.

An examination of a large number of injured plants showed that the deeper roots had been killed (Fig. 1, B). Since the roots were very short

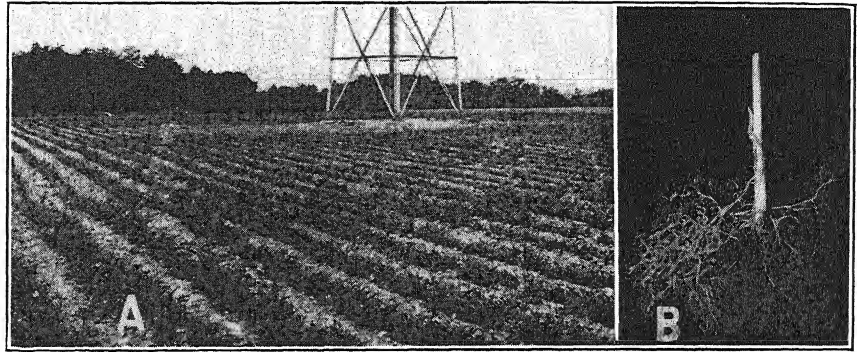


FIG. 1. Lightning injury of black locust seedlings. A. A general view of the damaged spot that extended from one side of the power-line tower. B. External view of an injured seedling showing the killed roots and the root growth that was stimulated after the injury.

and still succulent when the injury occurred, the killed tissues simply collapsed and did not show any marked internal symptoms. Jones and Gilbert¹ reported a case of lightning injury of tobacco where the roots were killed and had a charred appearance. There were no external symptoms of injury on the stems or leaves of the locust seedlings. Isolations from the injured roots did not yield any of the known parasitic fungi. Root growth had been stimulated above the point of injury on many of the survivors (Fig. 1, B) that were examined about 2 weeks later. Walker² reported that adventitious roots were stimulated above the point of injury on the stems of cabbage that had been injured by lightning. Most of the severely injured plants stayed dwarfed and more or less chlorotic, and either died ultimately or were considered unusable for planting purposes. This case of lightning injury is of particular interest because it may explain some of the sudden occurrences of seedling losses in fairly large spots in forest nurseries.—L. W. R. JACKSON, Division of Forest Pathology, Bureau of Plant Industry in cooperation with the Allegheny Forest Experiment Station and the University of Pennsylvania, Philadelphia, Pa.

An Attempt to Propagate Tobacco-mosaic Virus 1 in the Chorio-allantoic Membrane of the Developing Chick Embryo.—The chorio-allantoic membrane of the developing chick embryo has been found unusually favorable for the propagation of many different viruses affecting animal hosts. Even some viruses not infective for chicken or bird have been shown to multiply without difficulty on or in the embryo. Since attempts to propagate viruses of plant origin on this living animal medium have not yet been reported, it is the purpose of this paper to describe an experiment in which tobacco mosaic virus 1 has been used to inoculate the chorio-allantois.

Leaves from Turkish tobacco plants showing typical tobacco-mosaic symp-

¹ Jones, L. R. and W. W. Gilbert. Lightning injury to herbaceous plants. *Phytopath.* 8: 270-282. 1918.

² Walker, J. C. Injury to cabbage by lightning. *Phytopath.* 27: 858-861. 1937.

toms were frozen, passed through a meat grinder, and the juice expressed by wringing the macerated tissue in a piece of cheesecloth. The extracted juice was centrifuged and purified by Stanley's ammonium sulphate-celite method and then passed through a Berkefeld "V" filter. Fresh fertile chicken eggs were incubated for 9 days at 40° C. and prepared for inoculation, using Burnet's¹ modification of Woodruff and Goodpasture's² method as recommended by Professor Jacob Traum and Miss Miriam Smith of the Division of Veterinary Science.

Five eggs were inoculated by gently swabbing a sterile cotton pellet soaked with the purified virus suspension, over the chorio-allantois. Five other eggs were inoculated in a similar way, but by first touching the wet pellet to a small quantity of carborundum powder before inoculating the membrane. By using the carborundum powder it was hoped that some of the virus would be introduced directly into the cells of the membrane through the abraded surface. After incubating the inoculated eggs for 3 days at 37° C., the exposed portion of chorio-allantoic membrane was removed from the living embryos. A small piece of it about 0.3 cm. square from each egg was used to inoculate a succeeding set of incubated eggs. The remaining piece of each membrane was ground in a porcelain mortar and 2 cc. of distilled water were added to make sufficient volume to inoculate 5 half leaves of *Nicotiana glutinosa*. The other half of each leaf was inoculated with the filtered purified virus. Four successive subplants were made on incubated eggs. In no case were local lesions formed on leaf halves inoculated with the egg membrane material, while opposite halves inoculated with filtered purified virus showed an average of 250 spots per half leaf on *N. glutinosa*.

Under the conditions of these experiments, not only did tobacco-mosaic virus fail to multiply in the chorio-allantoic membrane but active virus could not be detected by the local-lesion method in the membrane on which the original virus inoculation had been made.—WILLIAM N. TAKAHASHI, Division of Plant Pathology, University of California, Berkeley, California.

Carborundum for Plant-virus Inoculations.—Following the development of the carborundum method,^{1, 2} numerous inquiries relating to the grade and quality of powdered carborundum have been received. It has seemed desirable, therefore, to present more complete information regarding the carborundum that has given satisfactory results during the past 5 years.

Powdered carborundum, 600 mesh, is obtainable in any quantity from Braun-Knecht-Heimann Company, San Francisco, California. When ordering, it is advisable to refer to their stock number 38713. Carborundum in-

¹ Burnet, F. M. A virus disease of the canary of the fowl-pox group. Jour. Path. 37: 107-122. 1933.

² Woodruff, Alice Miles and Ernest W. Goodpasture. The susceptibility of the chorio-allantoic membrane of chick embryo to infection with the fowl-pox virus. Amer. Jour. Path. 7: 209-222. 1931.

¹ Rawlins, T. E. and C. M. Tompkins. The use of carborundum as an abrasive in plant-virus inoculations. (Abstract) Phytopath. 24: 1147. 1934.

² Rawlins, T. E. and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant-virus inoculations. Phytopath. 26: 578-587. 1936.

variably contains moisture upon arrival from the distributor; in this condition the powder is dark gray, and the particles adhere to each other in small to large aggregates. In this condition it cannot be used to advantage in a salt shaker. It is suggested, therefore, that, when received, the lid of the container be removed and the carborundum placed in a dry heat sterilizer at 80° to 90° C. for several hours, or until thoroughly dry. Drying restores the powdery condition and the light gray color of this particular grade of carborundum. If stored in tight-stoppered receptacles, no further drying is necessary.—T. E. RAWLINS and C. M. TOMPKINS, Division of Plant Pathology, University of California, Berkeley, California.

BOOK REVIEW

COUCH, J. N. *The Genus Septobasidium*. 473 pages, including 114 plates, 60 text figs. The University of North Carolina Press (Chapel Hill). 1938.

The pathologist encountering this thorough and significant monograph probably will begin with the Pathological Considerations and Control Methods of Chapter 2. Here his interest will be aroused at once by the statement that all species of *Septobasidium* cause damage to the trees on which they grow, the damage, with the extent of growth, varying from considerable to slight, numerous small trees of *Fraxinus* and *Nyssa*, for example, being killed outright by *Septobasidium pseudopedicellatum* and *S. curtisii*, while large trees, heavily infested, are very unhealthy with many dead limbs. Continuing, he will note with interest that the commonest type of injury involves chiefly cracking of the bark, in some cases with girdling of the branches or smaller trunks, and secondarily the formation of witches brooms, such injury occurring not only in the United States, particularly in the South, on such trees as ash, maple, holly, magnolia, pear, apple, etc., but in the American and Oriental tropics on citrus, acacia, tea and others. Reading farther he will note with regret the failure of spraying as a control measure and with approval the success of the direct application of kerosene emulsion paste and of pruning infected branches during the dormant season. Then, as the pathologist reaches the end of the chapter, he will find, to his disappointment, that in Dr. Couch's opinion there is no immediate cause for alarm over *Septobasidium*, for although widespread and although abundant in certain localities, the trees to which it is injurious are not important timber, while on cultivated fruit and nut trees it is serious and abundant only when favored by neglect and poor conditions, not in well kept orchards. Nor will the reader be comforted by the author's final admonition that the *Septobasidium*-scale insect combination must be regarded as distinctly harmful.

At this point it is possible that the young pathologist, conditioned by his previous training to believe that information of value to him must follow the stereotyped pathological pattern with which he is familiar, and inured to a complacent mental myopia, may be inclined to read no farther leaving the remainder of this significant study to the impractical mycologist or the omnivorous entomologist.

Let me assure the young pathologist that in the remaining 476½ pages of this unusually able work he will find that *Septobasidium* offers much of interest and value, even though it may not occasion any remunerative fellowships or become the objective of well financed state or federal campaigns of eradication. The older pathologist does not need this assurance for he has long since realized that basically underlying the practical aspects of plant pathology are such fundamental problems as parasitism and interaction, problems of biological significance in themselves and often of practical value in their bearing on control.

Of especial interest is the well worked out study of the intricate Fungus Insect Relationship in Chapter 1. The scale insects provide food and a means of distribution for the fungus, while the *Septobasidium* furnishes protection and a home for the insects. Of this two-membered consocium it is the insects that damage the underlying tree by their suctorial tubes, which pierce through the bark to the cambium, the fungus contributing indirectly by fostering the insects, but doing little damage directly since in only a few species may the hyphae penetrate slightly into the living tissue of the bark or leaf. The relation involves an unusual nicety of balance, the insect colony, through the negligible sacrifice of the relatively few individuals parasitized by the fungus gaining as a whole an effective protection and a specialized housing, which fosters the production of offspring and facilitates their escape to accomplish the dispersal of the fungus by carrying its spores. The association is thus one of mutual benefit, a symbiosis in the

strict sense of the word, and the biological situation involved, an intersection of mycology, entomology and plant pathology, leads into such fascinating paths of speculation as the evolutionary origin of such a specialized association so obviously of long standing. Furthermore, this combination of insect and fungus results in a new type of organism differing from either participating entity by itself and hence leads into the realm of emergent evolution in the borderland of philosophical biology remote from most of us but explored to the enrichment of biologic thought by such intrepid souls as Wheeler.

The associative complex here involves a large number of scale insects, the 54 species identified from United States material and the 45 known from other sources, belonging chiefly to the genus *Aspidiotus*, some to *Chionaspis* and *Chrysomphalus*, with a few scattered through several other genera, probably representing only a part of the actual representation. The fungous participant, *Septobasidium*, comprises around 170 species of which about half have been established by Couch in his thorough twelve years' study of living and herbarium material in this country, Europe, and the West Indies.

Especially interesting and valuable is the discussion of the geographic distribution of *Septobasidium* so far as it is known, listing the number of species collected from various countries and interpreting both the general distribution and some of the more notable special cases on the basis of such chief means of dissemination as the spread of infected young scale insects, whether through their own crawling or through being carried by birds, by other insects or by the wind, and the spread of established *Septobasidium*-insect communities through transplantation or long distance transport of the host trees. Of interest also is the detailed analysis of the distribution of *Septobasidium* in the United States and Canada with its effective tabulations of distribution by States according to the number of collections not only for the several states but also for the various host trees and its analyses of the special points of interest involved.

Especially thorough and significant also is the detailed account of the structure, development, and reproduction of the fungus with a careful comparative study of the specialized features of the traps, tunnels, houses, and breeding chambers fostering the insects and of the haustoria parasitizing them.

With this comprehensive study as a foundation, the structural features of taxonomic importance are carefully evaluated serving as a sound basis for the 230 pages of the taxonomic section with its revised and extended generic description, its effective key to the species, its detailed specific descriptions and its supplementary notes on species incompletely known or justifiably excluded.

The relation of *Septobasidium* to other members of the lower Basidiomycetes with transversely septate basidia and especially to the rusts is carefully considered and the genus established as an order, Septobasidiales, of the Hetero-basidiomycetes, coordinate with the Auriculariales, Uredinales and Ustilaginales. Questions of cytology and hybridization are covered briefly but adequately, while a comprehensive reference list of 120 titles, thoroughly covering the literature and a serviceable and extensive index complete the text.

The excellent and numerous illustrations fittingly complement the thorough and comprehensive text. Assembled in the 48 half-tone plates are numerous photographs showing the characteristic habit and distinctive gross structural features of all the more important species, while the zinc cuts, wash drawings, and photographs of the frontispiece, the 60 text figures and the first 66 plates illustrate with admirable effectiveness the structure of the participating insects themselves, their development in relation to the host trees and to the traps, breeding chambers and other specialized structures of the fungus, and the context, hymenium, basidia, spores, and other distinctive morphological details of the several species of *Septobasidium*.

The book is a monograph worthy of a place beside that of Thaxter, an inspiration to the mycologist and plant pathologist, a significant contribution to biology as a whole.

* * * * *

Since this review was written, Dr. Couch's work has been awarded the Walker Prize of the long established Boston Society of Natural History, a signal honor, as this prize is given in recognition of the most outstanding contribution, whether a single piece of work or a concerted program, in the field of Natural History during the preceding five years.—WILLIAM H. WESTON, Biological Laboratories, Harvard University, Cambridge, Mass.

THREE SPECIES OF PYTHIUM ASSOCIATED WITH ROOT ROTS

CHARLES DRECHSLER

(Accepted for publication October 7, 1939)

In a paper (9) published nearly 10 years ago I presented as new 15 species of *Pythium* that had been isolated from decaying parts of various phanerogamic host plants originating from different localities in eastern and southern regions of the United States. Aside from some introductory comments, mainly of a comparative nature, the descriptions then accorded to the new forms were limited to diagnostic statements not accompanied either by needful explanatory remarks or by figures illustrative of details and peculiarities difficult to set forth adequately in words. It is hoped that as far as 6 of the species are concerned, these deficiencies of treatment have in a measure been remedied in supplementary accounts (12, 13) that have recently appeared in this journal. Similar supplementary consideration is herein devoted to 3 additional species, *P. dissotocum*, *P. peritum*, and *P. paroecandrum*, all of which I described from pure cultures isolated through procedure elsewhere (7, p. 310-312) recorded, from softened or discolored tissues of roots affected by decay. To facilitate comparison, the accompanying figures were prepared for reproduction at magnifications uniform with those in similar illustrations of the congeneric forms dealt with earlier. As the zoosporangia of *P. paroecandrum* are of the conveniently compact sub-spherical type and appear unaccompanied by significant differentiation of supporting hyphae, they are shown, like some of the less rangy homologous structures (12, p. 399, fig. 3, I, J, K) of *P. acanthicum* Drechsl., at the same magnification (*i.e.*, $\times 1000$) employed in illustrations of sexual apparatus, rather than at the lower magnification (*i.e.*, $\times 500$) resorted to in figures of the more extensive filamentous or lobulate sporangial units of numerous related species.

PYTHIUM DISSOTOCUM

The diagnosis of *Pythium dissotocum* Drechsl. was based primarily on a culture submitted to me in a varied assortment of fungus cultures isolated by R. D. Rands from diseased roots of sugar cane, *Saccharum officinarum* L., collected near Thibodaux, Louisiana, in April, 1927; some utilization, however, being made also of observations on 5 other cultures in the same assortment that were easily recognized as conspecific from a thoroughgoing similarity of macroscopic appearance, and from a close parallelism in mycelial habit, zoosporangial development, and arrangement of sexual apparatus revealed by each of them under the microscope. On diseased sugarcane roots the fungus would seem to occur with moderate frequency. Under the binomial *P. dissotocum*, Rands and Dopp (19) make mention of 57 cultures that were isolated by them from such roots and subjected to growth measurements and to tests for pathogenicity. They further cite *P. dissoto-*

cum among the 3 species that, apart from *P. arrhenomanes* Drechs., were most frequently obtained by them from rotted sugar-cane roots in 1930. Inoculation experiments of these authors reveal the fungus as only weakly parasitic when environmental conditions are in ordinary degree favorable for the host plant; severe root rot with appreciable reduction in plant weight ensuing, however, under the predisposing influence of a soil toxin, salicylic aldehyde, in dilute concentration.

The fungus is known to occur also on phanerogamic plants other than sugar cane. It was found in several sets of cultures isolated from softened roots of canning peas, *Pisum sativum* L., collected in the course of a survey on which a report (5) was rendered in 1925. The sets of cultures in question were derived from collections made respectively near Easton, Maryland, May 15, 1924; near Centerville, Md., May 16, 1924; near Bridgeville, Delaware, May 27, 1924; near Cedarville, New Jersey, May 29, 1924; and near Westminster, Md., June 11, 1924. *Pythium dissotocum* was likewise represented in 2 cultures isolated by F. R. Jones from pea roots collected in the course of another disease survey (16). One of these cultures was derived from material collected near Templeton, Wisconsin, July 4, 1924; the other from material collected near Gillett, Wis., July 17, 1924. The fungus was obtained later from discolored rootlets of *Pilea pumila* (L.) Gray collected near Cabin John, Md., Oct. 20, 1926. Four cultures isolated from discolored rootlets of the sugar beet, *Beta vulgaris* L., gathered near Saginaw, Michigan, late in June, 1927, have been readily identified as belonging to the species; and a similar determination was made of 3 cultures derived from discolored roots of spinach, *Spinacea oleracea* L., collected near Norfolk, Virginia, late in November, 1932.

In pure culture on a transparent gel substratum not excessively rich in nutrients, such as is available more especially in maize meal agar, *Pythium dissotocum* grows appreciably more slowly than the very familiar congeneric forms causing damping-off in seed-beds. Aerial mycelium is usually altogether absent on this medium, though sometimes developing in meager quantity on substrata that contain food substances in higher concentrations. As was stated in the diagnosis, the submerged mycelium is somewhat lustrous, presenting an appearance, therefore, in a sense median between that of the diffuse intramatrical mycelium of *P. ultimum* Trow on the one hand, and that of the very lustrous mycelium of *P. complens* Fischer on the other. Commensurate with its moderate luster, the thallus of *P. dissotocum*, while consisting more largely than that of *P. ultimum* of rather straightforward axial hyphae arranged nearly parallel with one another, is composed of such hyphae in smaller measure than is the thallus of *P. complens* or of the similarly very lustrous *P. vexans* de Bary (= *P. complectens* Braun). The curious regional variegation with respect to density of hyphal development that becomes apparent to the naked eye in a cumulous effect is not usually evident in cultures of the fungus under consideration. Appressoria of modest

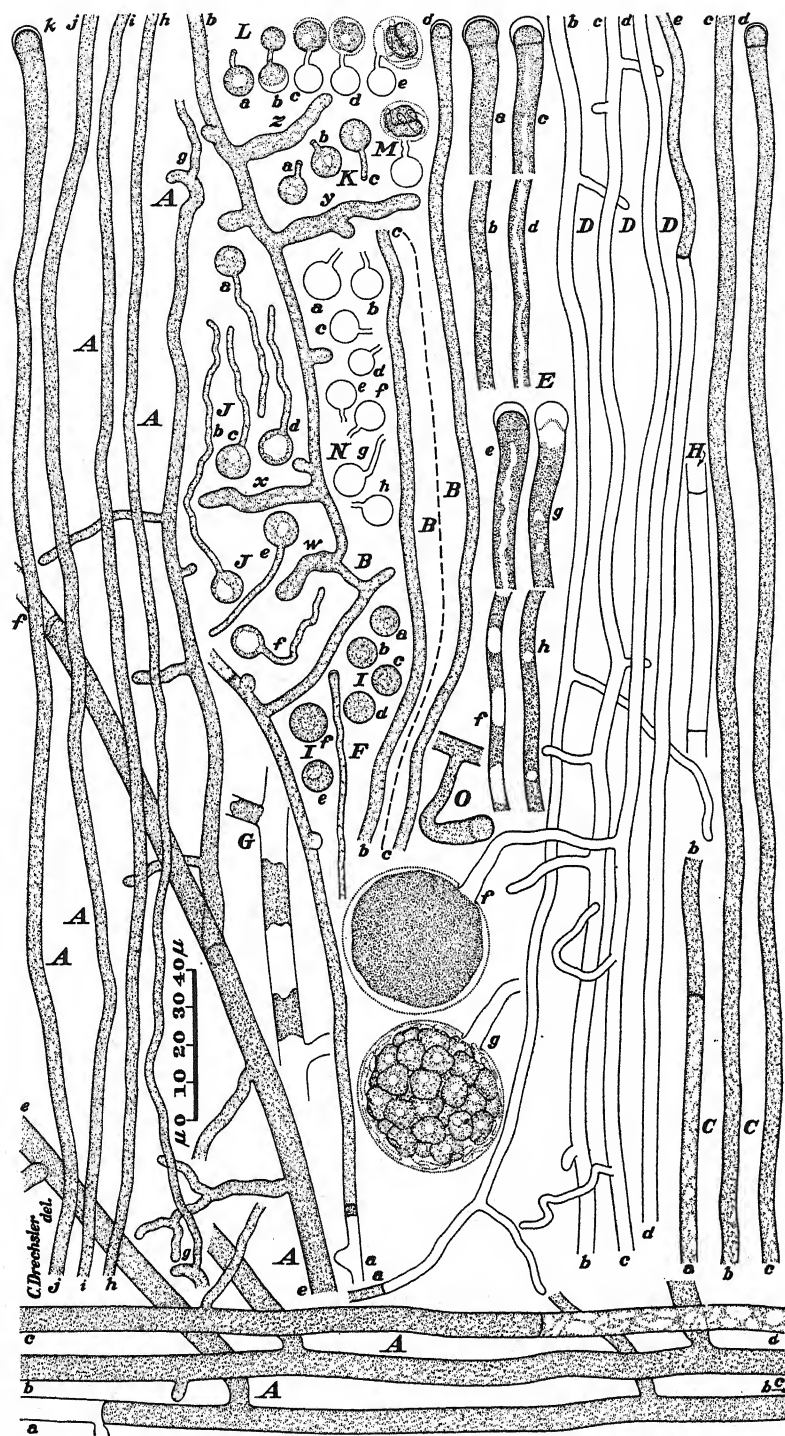


FIG. 1. Asexual reproductive apparatus of *Pythium dissotocum* Drechs. drawn with aid of a camera lucida; $\times 500$. From lack of space A is shown in sections connecting at the points b, c, e, g, h, i, j; B similarly connecting at points b, c; C connecting at points b, c; and D connecting at points b, c, d.

dimensions (Fig. 1, O) are formed in some numbers where hyphae come in contact with the surface of the culture dish or of other hard objects.

Production of zoospores by *Pythium dissotocum* ensues with much regularity when vigorous mycelium of the fungus is bathed in water. Extraordinarily prolific development of swimming spores may be induced conveniently by cutting sizeable slabs well permeated with young mycelium from a vigorously growing Lima-bean agar plate culture, then transferring the excised slabs to an empty sterile Petri dish, and irrigating them by careful addition of well-aerated sterile water until the upper surface of the substratum is barely flooded. Often, on proper manipulation, virtually the entire mycelium becomes converted into sporangial units. In many instances the individual unit is composed of a longish portion of a wide axial filament together not only with contiguous portions of a few main branches but also with perhaps more numerous narrower lateral ramifications, one of which may become prolonged into a rangy evacuation tube more than 1 mm. in length (Fig. 1, A, a-k). Such a large extensive sporangium naturally gives rise to a correspondingly large vesicle wherein from 100 to 125 zoospores may be fashioned. A sporangial unit of more moderate volume consists often of an intercalary portion of filament, 1 to 2 mm. long and 3 to 4 μ wide, together with a half-dozen subsidiary ultimate branches, and yields between 50 and 75 zoospores (Fig. 1, D, a-e, f, g). Frequently an unbranched terminal portion of filament, from 0.5 mm. to 1 mm. in length, becomes delimited by a basal septum, and, after forming a broad tip of dehiscence (Fig. 1, C, a-d), functions as a sporangium. A small sporangium may be provided with an evacuation tube measuring as little as 1.5 μ in width below an expanded tip only 2.5 μ wide (Fig. 1, F).

Under conditions very favorable for zoospore production, conversion of a portion of vegetative mycelium into an asexual reproductive unit is in most instances not preceded or accompanied by development of any specially differentiated elements, apart, of course, from the expanded cap of dehiscence. In less numerous instances (Fig. 1, B, a-d), however, such conversion entails production of several swollen lateral branches (Fig. 1, B, w-z) noticeably wider than the filament bearing them, though frequently not exceeding in width the undifferentiated main axial hyphae of the fungus. The swollen branches attain more conspicuous development ordinarily after an expanse of mycelium has largely exhausted itself in zoospore production and has possibly been affected besides by some accumulation of staling products, as well as by incipient bacterial contamination (Fig. 2, A). Though usually affording only a rather meager display, the dactyloid branches appear truly homologous with the distended digitations, lobulations, and moriform aggregations familiar in certain congeneric forms like *Pythium arrhenomanes*, *P. complens* and *P. periplocum* Drechsl.

The zoospores of *Pythium dissotocum*, after swimming about very actively for a variable period, come to rest and round up, thereby forming spherical cysts slightly smaller than the cysts of *P. debaryanum* Hesse and of most

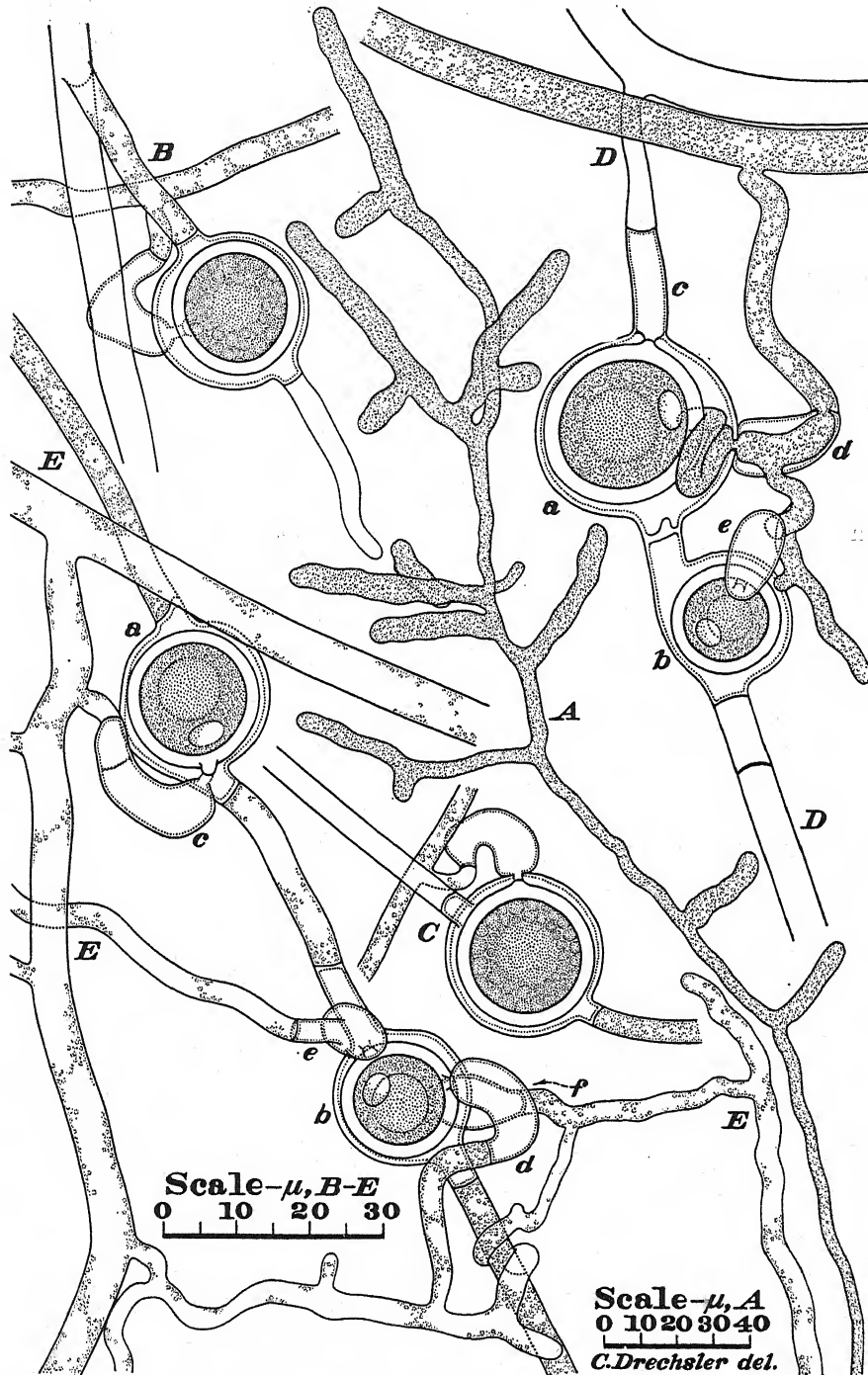


FIG. 2. *Pythium dissotocum* drawn with the aid of a camera lucida. A. Portion of irrigated mycelium, showing zoosporangial differentiation in relatively luxuriant development of inflated lateral branches; $\times 500$. B-E. Units of sexual apparatus; $\times 1000$.

other allied species familiar to plant pathologists. Often the spherical bodies then germinate in a commonplace manner by the production usually of a single delicate germ hypha (Fig. 1, J, a-f); or, again, they may develop iteratively, each putting forth an evacuation tube (Fig. 1, K, a-c; L, a) and discharging its contents (Fig. 1, L, b) into a small vesicle, there to be fashioned into a new biciliate motile zoospore (Fig. 1, L, c-e; M) of the same type as the one from which it originated. As a result of such iterative development, innumerable empty cyst envelopes with open evacuation tubes of varying lengths (Fig. 1, N, a-h) are often to be observed scattered about everywhere in an irrigated preparation.

The frequency of iterant swarming in zoospores of *Pythium dissotocum*, to which brief allusion was made in an earlier paper (8, p. 569, lines 47 to 50) devoted mainly to similar activity in zoospores of various other pythiaceae forms, suggested the specific epithet, a term meaning "twice-born," subsequently applied to the fungus. Additional instances of iterant swarming following repeated emergence have since been supplied by Sparrow (21) in the descriptions of his *P. adhaerens* and his *P. angustatum*. Höhnk (14) noted that the zoospores of the fungus he described as *P. epigynum* underwent a second swarm period when fresh water was added after a first encystment had occurred. This investigator later took occasion to give details concerning particular examples of repetitional development observed by him (15).

Most strains of *Pythium dissotocum* ordinarily show abundant and prompt sexual reproduction when grown on maize meal agar containing in suspension a moderate quantity of finely ground maize meal. It is true, sexual reproduction occasionally fails to take place, not only in the more refractory strains but also in strains habitually productive of oospores in immense numbers. As the conditions evoking such apparently capricious behavior have not hitherto been determined, it may only be conjectured that possibly some specific nutrient substance, not always available in sufficient quantity, exerts a governing influence. However, once sexual structures have been formed, they are little given to degeneration on a serious scale.

The subspherical oogonia of the fungus may be terminal (Fig. 3, A; D, a; H) or subterminal (Fig. 2, B; Fig. 3, E) on branches of variable length, though more often they are borne on the main hyphae in intercalary positions, sometimes mesially (Fig. 2, C; D, a; E, a, b; Fig. 3, B; C; D, b; F; J; K, b; L, a) or, again, laterally (Fig. 3, G, a, b; I; K, a; L, b). As the delimiting septa are often placed at appreciable distances from the subspherical contour, cylindrical parts, commonly 2μ or 3μ long, but sometimes measuring 5μ (Fig. 3, J), 6μ (Fig. 3, C), 7μ (Fig. 3, D, b) or even 11μ (Fig. 2, E, b) in length, come to be included in the female organ. Not infrequently, 2 intercalary oogonia are formed adjacent to each other (Fig. 2, D, a, b; Fig. 3, G, a, b; L, a, b).

The male complement of the individual oogonium consists usually of 1 to 3 antheridia, which, for the most part, are of the inflated crook-necked type

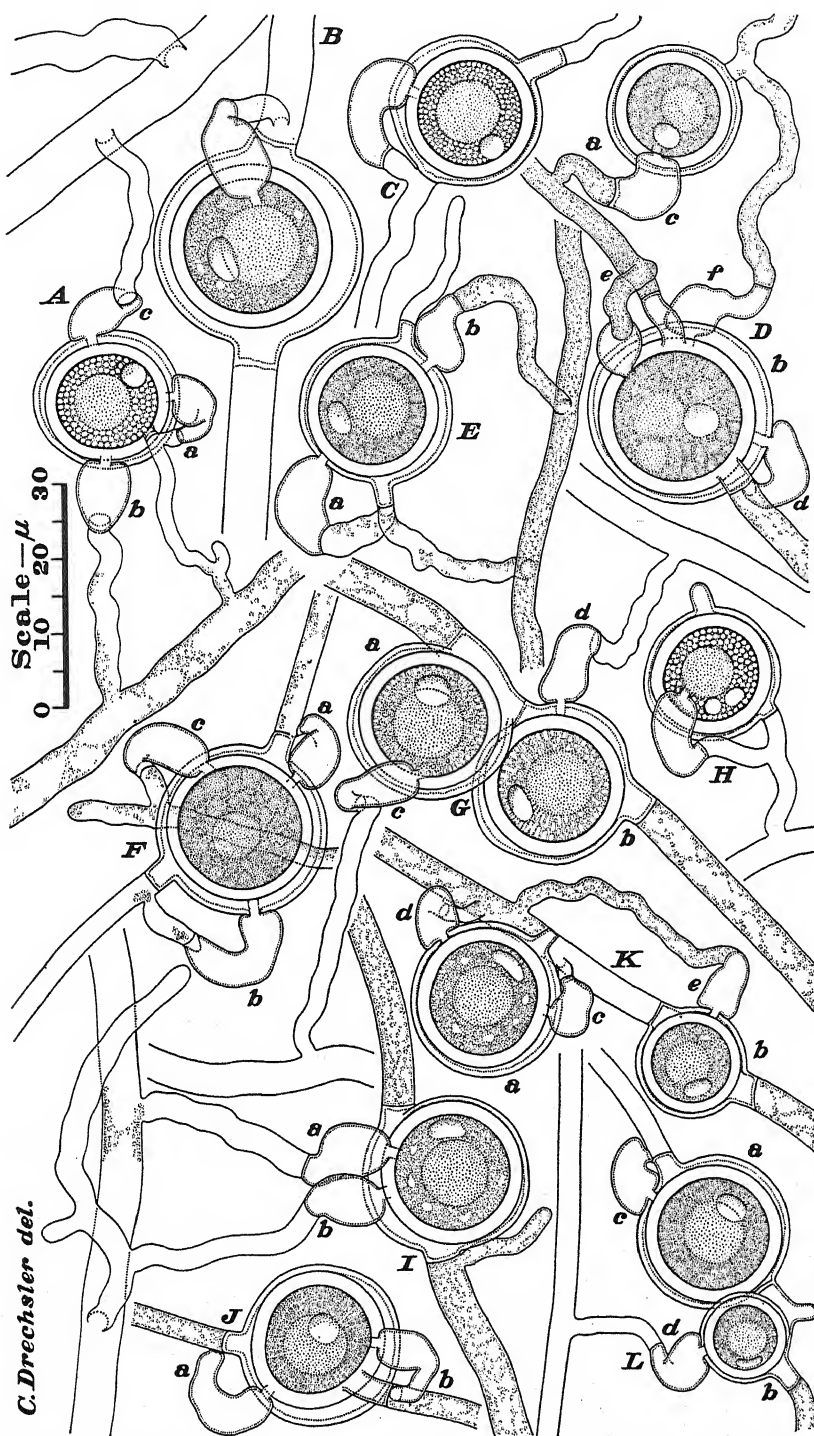


FIG. 3. Sexual apparatus of *Pythium dissotocum* drawn with the aid of a camera lucida to a uniform magnification; $\times 1000$ throughout.

familiar in many congeneric species. Where plural antheridia are present they appear usually to have arisen without reference to one another, often despite a moderately close mycelial connection between them. As for their other relationships, the antheridia are often borne terminally on branches arising either from neighboring hyphae having no close connection with the oogonial filament (Fig. 2, B; C; D, e; E, e, d, f; Fig. 3, A, c; D, c; F, c; G, c, d; I, a, b; L, d), or from the oogonial filament at variable distances from the oogonium (Fig. 3, A, b; E, b; H; K, c, d, e), or often, again, from the oogonial filament in close proximity to the oogonium (Fig. 3, A, a; B; C; D, e; E, a; F, b). Often, too, an antheridium is borne sessile on the oogonial filament in immediate proximity to the oogonium (Fig. 3, D, d; F, a; J, a, b; L, e). The "hypogynal" type of antheridium, consisting of an outwardly undifferentiated portion of filament adjacent to the oogonium (Fig. 2, D, c) has likewise been observed in cultures of the fungus, though so infrequently that the few instances must be regarded as somewhat exceptional.

Application of the antheridium to the oogonium usually entails some apical flattening, so that contact between the opposed organs is generally wider in this species than in *Pythium peritum*, for example. In units of sexual apparatus with plural antheridia, all ordinarily discharge their contents into the oogonium. As in many other fungi, various irregularities of sexual reproduction occur in *P. dissotocum*, an example being illustrated somewhat incidentally in figure 2, D, where the antheridium "d," which presumably became applied to the oogonium "a" at a relatively late stage, is shown to have resorted to vegetative growth by intruding a hyphal prolongation into the unoccupied portion of the oogonial chamber, and by putting forth laterally a filament that gave rise to a short branch bearing the antheridium "e," which clearly was operative in the fertilization of the oogonium "b."

Following fertilization the oogonium of *Pythium dissotocum* shows a sequence of internal change familiar in most congeneric species. The contracted protoplast surrounds itself with a thick wall. The sizeable lumps of somewhat homogeneous consistency into which the porridge-like granular material has been aggregated, become displaced at the center of the young sexual spore by a homogeneous globule of increasing size (Fig. 2, B; C; Fig. 3, F). The resulting parietal layer diminishes in thickness correspondingly, and often reveals a perceptibly radial orientation as its constituent lumps undergo transformation into smaller granules (Fig. 3, D, a, b; E; G, a, b; J). At early maturity the layer has a densely and rather minutely granular texture, contrasting sharply with the apparently homogeneous structure of the single subspherical or oblate ellipsoidal refringent body (Fig. 2, D, a, b; E, a, b; Fig. 3, B; I; K, a, b; L, a, b). After several weeks of aging it is often found composed of larger subspherical granules measuring about $.5\mu$ in thickness (Fig. 3, A, C), and the single refringent body may be replaced by 2 similar bodies of slightly reduced dimensions (Fig. 3, H).

In the texture of its parietal layer the oospore at advanced maturity thus comes to present an engaging resemblance to the oospores of various Saprolegniaceae, including the several terrestrial parasitic species known to cause root rot in phanerogamic crop plants. This resemblance would seem sustained in a transitory arrangement of protoplasm observable in the zoosporangium of *Pythium dissotocum* shortly, though not immediately, preceding its evacuation. During the earlier stages in the development of an apex of dehiscence (Fig. 1, E, a), the contents of the hyphae to be included in the new sporangial unit show little alteration from the granular texture usual in vegetative filaments (Fig. 1, E, b). Later, when the hyaline cap has nearly attained its definitive proportions (Fig. 1, E, c), longitudinal vacuoles make their appearance in the hyphae, and often coalesce into extended axial lacunae of irregular outline (Fig. 1, E, d). In some of the narrower filamentous elements, though not usually throughout the sporangium (Fig. 1, E, e), the longitudinally vacuolate condition may for a brief time be supplanted by a transversely vacuolate one (Fig. 1, E, f), so that cylindrical portions of protoplasm alternate with regularly spaced vacuoles in a manner recalling the arrangement of zoospore protoplasts in *Aphanomyces* sporangia previous to their becoming connected by axial strands. To be sure, the transversely vacuolate condition is rather completely obliterated in the sudden reorganization of contents that precedes sporangial discharge by a few seconds. In this reorganization, which is often accompanied by a visible jolt of the hyphae concerned, the protoplasmic contents are withdrawn a short distance from the hyaline cap (Fig. 1, E, g), and revert throughout the reproductive unit to a granular texture relieved only sparingly by a few small, scattered vacuoles (Fig. 1, E, h).

An interrupted disposition of protoplasm, somewhat similar to that associated transitorily with sporangial development, is observable often in aging vegetative filaments (Fig. 1, G) of the fungus. Although aging of mycelium here entails much less abundant deposition of retaining cross-walls than in *Pythium debaryanum*, for example, successive septa may occasionally be found rather closely spaced in the empty hyphae (Fig. 1, H).

With regard to its principal dimensions, *Pythium dissotocum* shows the rather moderate range of variability prevailing in most members of the genus to which it belongs. The data in the diagnosis relevant to oogonial size were derived from 200 measurements of mature oogonia of obviously wholly normal development selected at random in maize-meal-agar cultures showing very copious sexual reproduction with virtually no degeneration. The 200 values for diameter of oogonium, expressed to the nearest micron, showed a distribution as follows: 12 μ , 1; 14 μ , 1; 15 μ , 3; 16 μ , 1; 17 μ , 3; 18 μ , 12; 19 μ , 34; 20 μ , 33; 21 μ , 43; 22 μ , 36; 23 μ , 17; 24 μ , 6; 25 μ , 7; 29 μ , 2; 32 μ , 1. Measurements of the 200 oospores of correct structure contained within the oogonia, gave the following values for diameter, expressed to the nearest micron: 11 μ , 2; 12 μ , 1; 13 μ , 1; 14 μ , 2; 15 μ , 8; 16 μ , 25; 17 μ , 48; 18 μ , 45; 19 μ , 42; 20 μ , 14; 21 μ , 6; 22 μ , 4; 26 μ , 1; 27 μ , 1.

PYTHIUM PERIILUM

The same collection of fungus cultures from sugar-cane roots that supplied the material on which primarily was based the description of *Pythium dissotocum*, included also the single culture from which, after varied treatment and propagation, was drawn the diagnosis of *P. periilum* Drechsl. Subsequently, a number of additional cultures, closely similar to the one in question with respect to macroscopic appearance, as well as with respect to morphology of sporangium and sexual apparatus, were committed to me by R. D. Rands and E. Dopp, who had isolated them likewise from affected roots of sugar cane in Louisiana. These investigators have found the fungus only feebly aggressive as a parasite, for under experimental conditions permitting severe damage by *P. arrhenomanes*, it caused only occasional root tips to become flaccid (19). In commenting on *P. periilum*, Stevenson and Rands (22) characterize the species as a weakly parasitic one, infrequently isolated from rotted rootlets of sugar cane.

On maize meal agar, *Pythium periilum* shows approximately the same rate of hyphal extension as *P. dissotocum*, and produces similarly an intramatrical mycelium with a lustrous radiate appearance expressive of a considerable degree of parallelism in the orientation of the main axial filaments. However, instead of the relatively uniform mycelial distribution usual in cultures of *P. dissotocum*, the vegetative thallus has rather marked local inequalities in the concentration of its hyphae, whereby it offers to the naked eye a patchy effect that from a suggestiveness of banked cumulous clouds was denominated "cumulous" in the diagnosis of the species.¹ Though aerial growth is usually absent in cultures on maize meal agar, some richer substrata as, for example, Lima-bean agar, sometimes afford meager development of aerial mycelium in a somewhat appressed, compact, felty layer.

On microscopic examination of its vegetative mycelium the fungus is revealed as one of the more delicate members of the genus to which it belongs. Knob-like appressoria of relatively small dimensions (Fig. 4, A, B) are formed in moderate numbers terminally on some of the delicate branches that encounter the bottom of a culture dish, or that otherwise come into contact with a hard object.

Asexual reproduction can be induced conveniently in *Pythium periilum* by excising sizeable slabs from a thinly poured Lima-bean-agar plate culture well permeated with young mycelium, and transferring them to a shallow layer of aerated sterile water in a sterile Petri dish. Some reproductive units are formed by direct conversion of undifferentiated filamentous hyphae, with only a rather meager increment accruing through development of an expanded tip of dehiscence. Sporangia of such meager external modification are, however, less frequent in irrigated material of the present fungus than in irrigated preparations of *P. dissotocum*, owing to a more abundant production of swollen digitate elements, here singly, there in some-

¹ Sideris (20) has since made reference to the same macroscopic effect by the descriptive term "rosette."

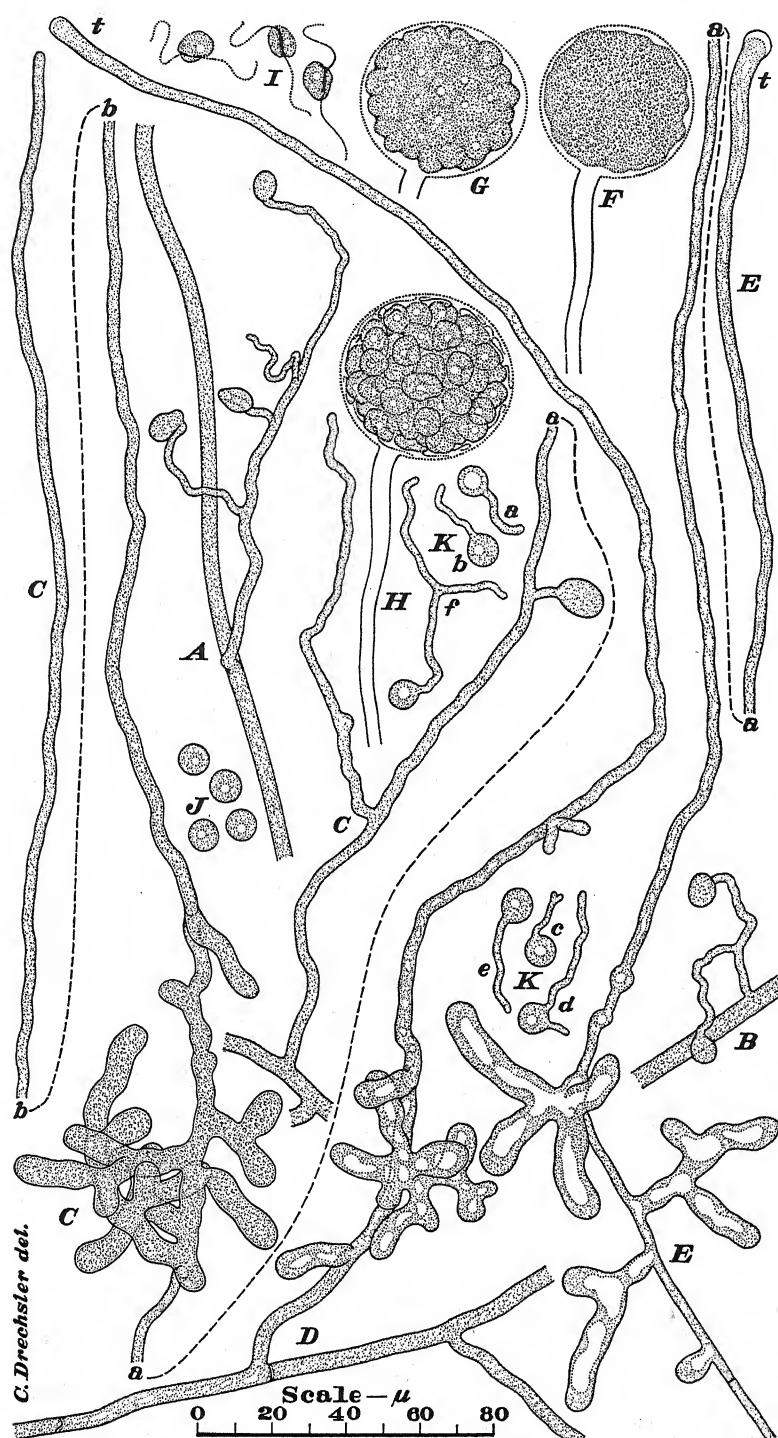


FIG. 4. Asexual reproductive apparatus of *Pythium peritum* Drechsler, drawn with aid of a camera lucida; $\times 500$. From lack of space C is shown in sections connecting at the points a, b; E similarly connecting at the point a.

what intricately branching systems (Fig. 4, C) comparable, more especially perhaps, with the homologous sporangial complexes of *P. myriotylum* Drechsl. Collectively, the swollen parts included in a sporangium, when it comes to be delimited by deposition of a septum (Fig. 4, E) or of plural septa (Fig. 4, D), often are of a volume equal to (Fig. 4, D) or exceeding that of the unmodified filamentous parts. As in other species vacuolization of the associated filamentous and swollen elements proceeds simultaneously with the development of a widened refringent apex of dehiscence on an evacuation tube frequently of considerable length (Fig. 4, D, t; E, t). Discharge of the sporangial contents into a vesicle resulting from inflation of the refringent cap (Fig. 4, F), transformation of the discharged mass into biciliate zoospores (Fig. 4, G, H), and liberation of the motile bodies (Fig. 4, I) by disintegration of the vesicular membrane, follow in familiar sequence.

With appropriate irrigation *Pythium peritum* produces swarm spores in about the same moderate measure of abundance as *P. myriotylum*; the fungus being in general more prolific asexually than *P. debaryanum*, *P. irregulare* Buism., and *P. mammillatum* Meurs, but appreciably less prolific than *P. butleri* Subr., and decidedly less prolific than *P. dissotocum*. After swimming about for some time the zoospores come to rest and round up into cysts (Fig. 4, J) that like the cysts of *P. dissotocum* would seem to be somewhat smaller than the homologous bodies of most of the congeneric parasites causing damping-off. Germination of the globose structures takes place mostly by production of a delicate germ tube (Fig. 4, K, a-c, e, f) or of 2 such tubes (Fig. 4, K, d).

In maize meal-agar cultures, containing some finely divided maize meal, *Pythium peritum* gives rise promptly to sexual apparatus that develops usually with little evidence of degeneration. The subspherical oogonia appear very often in intercalary positions (Fig. 5, A, D-L), less frequently in subterminal (Fig. 5, B) or terminal positions (Fig. 5, C). Generally, while the individual female organ is still continuous with its supporting hypha, it becomes inwrapped rather intimately and extensively by a branching filament (Fig. 5, A). Usually this filament arises from a hypha having no close mycelial connection with the one bearing the oogonium (Fig. 5, A-G, I-K), but occasionally it originates as a branch given off by the oogonial hypha at a distance perhaps not exceeding 50 μ from the female organ (Fig. 5, H). On the ramifications of this filament are soon borne, mostly terminally, but in some cases approximately laterally, from 2 to 5 antheridia, which become delimited by basal septa at about the same time the oogonium also is demarcated by deposition of one or, more often, 2 cross-walls, now flush with the spherical contour, now at a distance of 1 to 4 μ from it (Fig. 5, B-L). As a rule all of the antheridia discharge their contents into the oogonium, whereupon, if degeneration does not intervene, an oospore is formed that at early maturity shows the unitary internal organization evident in ripe oospores of most species of *Pythium*,—its single

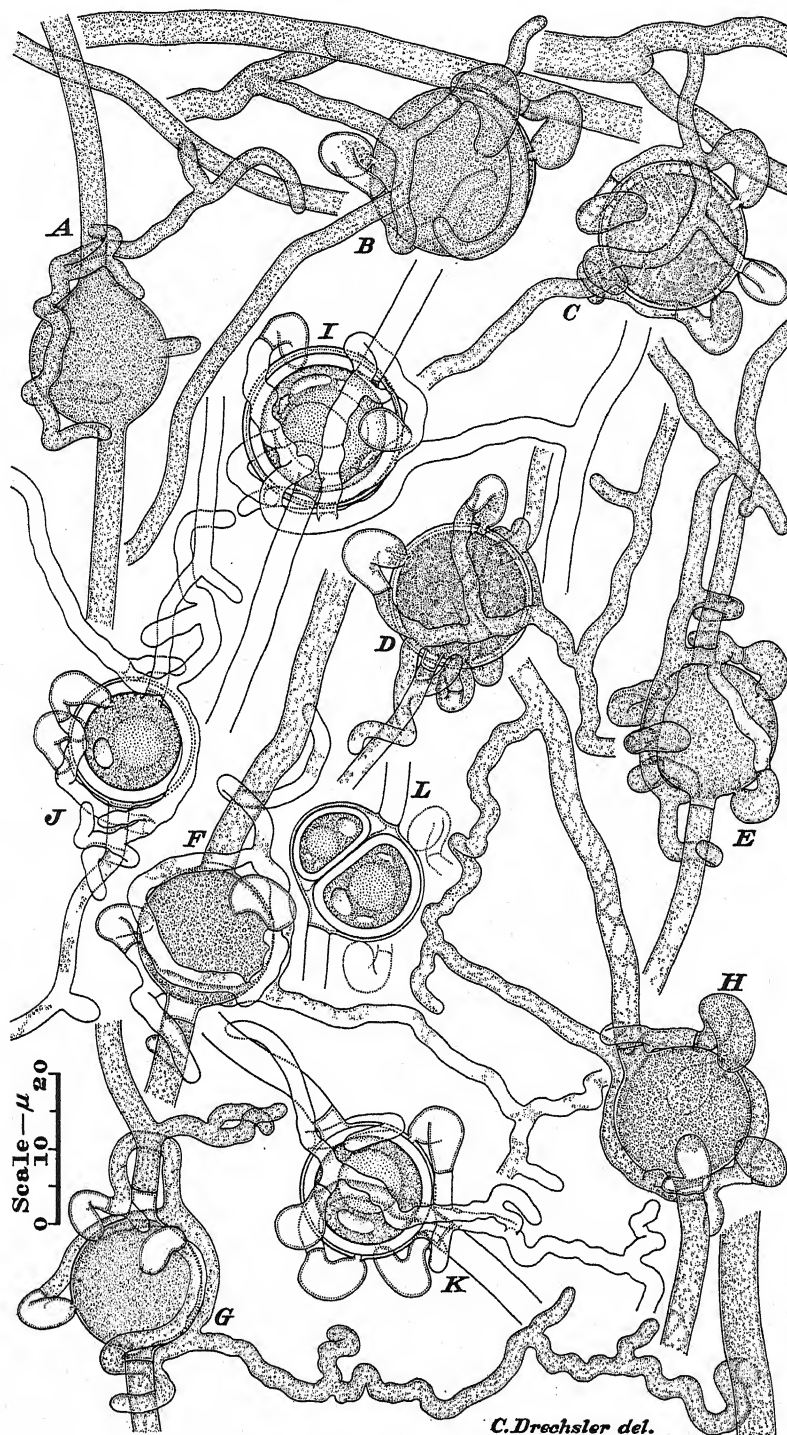


FIG. 5. Sexual reproductive apparatus of *Pythium peritum* drawn with the aid of a camera lucida to a uniform magnification; $\times 1000$ throughout.

central reserve globule being surrounded by a densely granular parietal layer in which is imbedded a single refringent body, subspherical or oblate-ellipsoidal in shape (Fig. 5, I-K). Developmental irregularity sometimes becomes manifest in the production of 2 oospores within an oogonium (Fig. 5, L). For better comparison with other species, all inordinately fecund units of sexual apparatus were excluded from consideration in the parts of the diagnosis relevant to the main dimensions of oogonium and oospore; the data there given having been derived from measurements of 100 monosporous sexual units selected at random in a maize-meal-agar culture that had produced oospores very abundantly with little evidence of degeneration. The 100 oogonia gave values for diameter, expressed to the nearest micron, with a distribution as follows: 16 μ , 1; 17 μ , 13; 18 μ , 23; 19 μ , 31; 20 μ , 22; 21 μ , 7; 22 μ , 3; and the oospores of correct unitary internal structure contained within them gave values for diameter, likewise expressed to the nearest micron, with the following distribution: 14 μ , 1; 15 μ , 2; 16 μ , 21; 17 μ , 32; 18 μ , 26; 19 μ , 14; 20 μ , 4.

Rather little diagnostic value attaches to the sizes of oogonium and oospore in *Pythium peritum*, as the homologous bodies of many congeneric forms, including the several species most commonly implicated in damping-off, reveal approximately similar dimensions. Certainly, much greater distinctiveness is represented in the extensive and close inwrapment of the female organ by the branching antheridial filament,—this feature, indeed, having suggested the epithet applied to the fungus, a term derived from a word meaning “to wrap round.” Inwrapment of equal extent and intimacy, though frequent among terricolous species of *Aphanomyces*, has been encountered elsewhere in the genus *Pythium*, as far as I am aware, only in *P. scleroteichum* Drechsl. (10), the parasite that, with *P. ultimum*, is responsible for mottle necrosis, a curiously labyrinthine decay of the edible roots of the sweet potato, *Ipomoea batatis* (L.) Lam. Similarity to *P. scleroteichum* is recognizable, besides, in a characteristic frail appearance of the rather small, thin-walled antheridia, which, like the thin-walled branches supporting them, become almost indiscernible after being evacuated of contents. However, the transverse dorsal furrowing, often to be seen in the antheridial branches of *P. scleroteichum*, has never been observed in *P. peritum*. Further, in *P. peritum* the oospore so nearly completely fills the oogonial chamber that, often in large part, its relatively thick wall appears more or less closely adnate to the much thinner, somewhat evanescent oogonial membrane; whereas, in *P. scleroteichum*, the oospore is always very loosely contained within a considerably larger oogonium, and its wall only slightly exceeds in thickness the conspicuous and extraordinarily enduring oogonial envelope.

PYTHIUM PAROECANDRUM

The diagnosis of *Pythium paroecandrum* Drechsl. was based primarily on a culture isolated from the somewhat blackened tip of a rootlet that alone

seemed to harbor an infection among hundreds of wholly unblemished rootlets on a flourishing clump of field garlic, *Allium vineale* L., originating from near McLean, Virginia, early in May, 1925. The culture in question had been the first one referable to the species to come into my hands. Previous to its description the fungus had been isolated also from several discolored rootlets of the pale touch-me-not, *Impatiens pallida* Nutt., taken from specimens of that plant collected in the District of Columbia early in September, 1926. A few additional conspecific cultures have since been obtained from separate discolored rootlets of the bloodroot, *Sanguinaria canadensis* L., collected in Arlington, Va., in April, 1931.

When planted on maize meal agar, *Pythium paroecandrum* gives rise to a slightly lustrous intramatrical mycelium of rather pronounced radiate appearance that extends itself about half as rapidly as mycelium of *P. ultimum*, *P. debaryanum* or *P. irregulare*. On this medium the fungus, unlike the 3 congeneric forms mentioned, produces usually no aerial hyphae, although on various richer substrata, such as Lima-bean agar, some meager aerial development may take place. When portions of a vigorously growing culture are removed to a shallow layer of water devoid of nutrients, hyphae are put forth into the surrounding liquid only a short distance and in relatively small quantity. In its feeble extramatrical development the fungus differs markedly from the species habitually associated with damping-off,—the difference connoting undoubtedly an incapacity on the part of *P. paroecandrum* to operate destructively as a seed-bed parasite, inasmuch as strong extramatrical development evidently constitutes not an incidental but an essential and necessary attribute of damping-off pathogens, enabling them to span readily the tracts of unnutritious soil separating individual seedlings from one another.

On appropriate irrigation fresh growth of *Pythium paroecandrum* rather promptly gives rise to subspherical zoosporangia. In dimensions and general conformation these bodies resemble approximately the zoosporangia of *P. debaryanum*, *P. irregulare* and *P. mammillatum*, though perhaps they more frequently include at one (Fig. 6, A, a) or both ends (Fig. 6, B) an outwardly unmodified hyphal portion between 5μ and 50μ in length. A sporangium with hyphal prolongations here, like the similarly composite asexual reproductive structures frequent in *P. acanthicum*, very often puts forth the evacuation tube from the cylindrical component (Fig. 6, A, t; B, t), rather than from the subspherical part. Indeed, even in instances where a hyphal extension is relatively short and of small volume, it yet serves frequently as origin of the evacuation tube (Fig. 6, C, t; E, t; F, t). The more purely subspherical sporangia usual in the species show little preference for any particular positional relationship of the evacuation tube (Fig. 6, G, t—O, t). With regard to vacuolization of the protoplasm within a sporangium, formation of a somewhat expanded hyaline cap at the tip of the evacuation tube (Fig. 6, B, t), discharge of the granular contents into a vesicle resulting from inflation of the cap, cleavage of the discharged

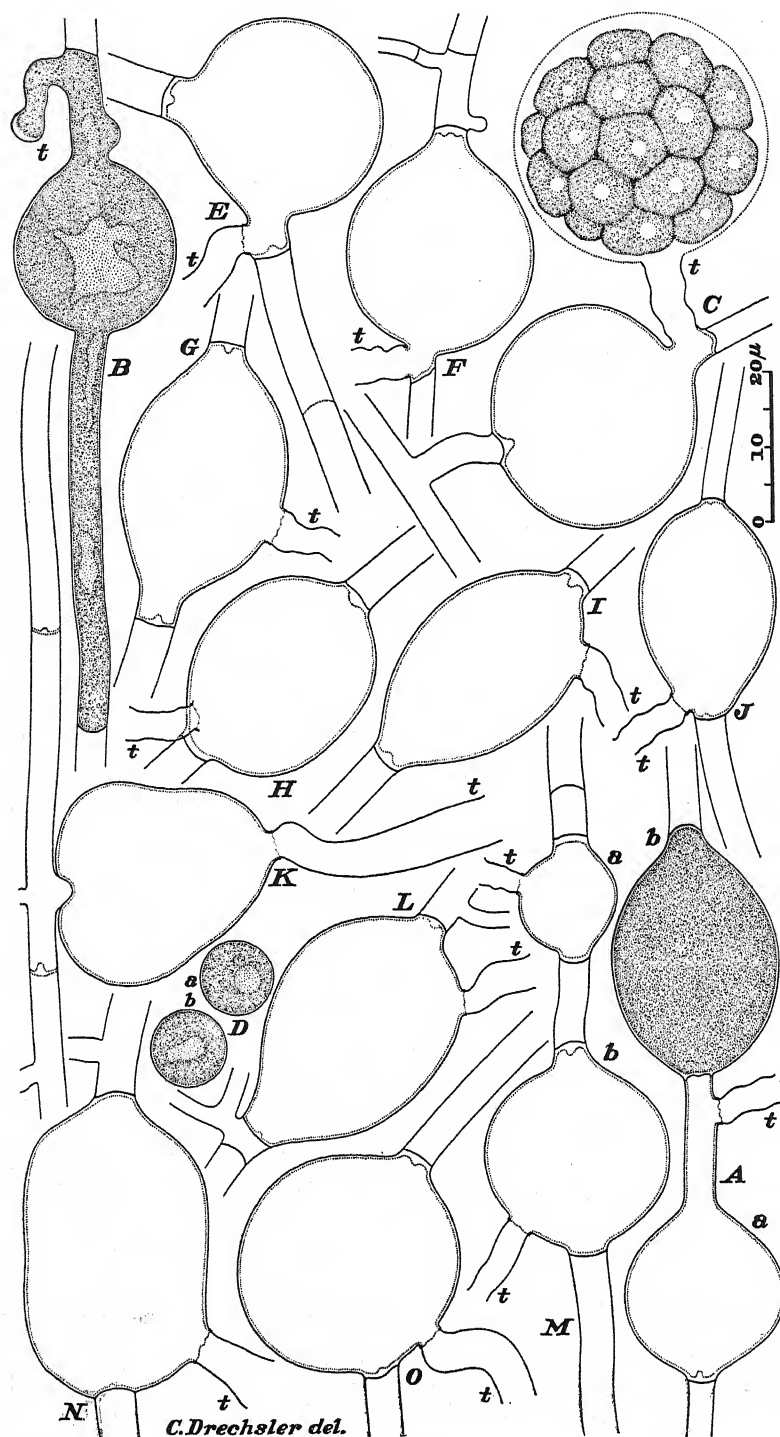


FIG. 6. Asexual reproductive apparatus of *Pythium paroecandrum* Drechs. drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout.

protoplasmic mass (Fig. 6, B), and its transformation into motile biciliate zoospores, the fungus shows general parallelism with *P. debaryanum*, *P. irregulare* and *P. mammillatum*. Its zoospores, like those of the 3 congeneric forms mentioned, round up into globose cysts (Fig. 6, D, a, b) slightly larger than the homologous bodies of *P. dissotocum* and *P. peritum*.

In maize meal-agar cultures *Pythium paroecandrum* forms sexual apparatus promptly and abundantly. The oogonia appear comparable to the asexual zoosporangia in their generally subspherical shape and predominantly intercalary position. They are supplied individually with 1 (Fig. 7, J; Fig. 8, A, f, g; B, d; D; E; F; G; I; O, c) to 5 (Fig. 7, C, c-g) antheridia. An antheridium often arises from a hypha having no close mycelial connection with the oogonial filament; but more frequently it originates from the oogonial hypha in close proximity to the oogonium. When of remote origin the male organ may consist of a saccate cell, borne laterally on an axial hypha (Fig. 8, D, I), or of a crook-necked inflated cell, borne terminally on a branch of varying length (Fig. 7, C, e, f, g); in neither case, however, revealing such variety and distinctiveness as when it arises in proximate relationship to the oogonium. The simplest type of antheridium contributed by the oogonial filament consists merely of an outwardly unmodified portion of the filament adjacent to the female organ (Fig. 7, F, a; Fig. 8, G; J, a; K, a). Such an antheridium, sometimes only 10 μ (Fig. 7, F, a), at other times over 25 μ (Fig. 8, J, a) long, of necessity thrusts its fertilization tube directly through the septum delimiting the oogonium, so that the cross-wall together with the tube make up a funnel-like protrusion, which, later, may frequently be seen with narrowed open end touching the oospore. Similar fertilization takes place in instances where, on conversion into an antheridium, the portion of hypha concerned undergoes slight external modification by becoming perceptibly distended at the end immediately adjacent to the oogonium (Fig. 7, B, a; L, a; Fig. 8, O, d; P, b). Further modification in antheridial shape, through the production of a lateral outgrowth arising always from a position close to the oogonium, permits intrusion of the fertilization tube through the spherical wall of the female organ. Depending on the measure of modification the lateral outgrowth may be of small volume in comparison with the cylindrical portion (Fig. 8, C, d; J, b); or, again, in instances where the cylindrical component is reduced to a very short segment, it may provide the main bulk of the antheridium (Fig. 7, D, b; J; Fig. 8, B, e; N, a). The latter condition approaches that represented in the frequent instances where the outgrowth is cut off by a basal septum to form by itself a male organ approximately sessile on the oogonial hypha (Fig. 7, B, b; D, a; E, c, d; H, a, b; I, f, g, h; Fig. 8, A, d; F; H, a, b; N, b; P, a). Often, especially when the outgrowth attains a somewhat greater length, the septum is laid down at an appreciable distance from the parent filament, with the result that the delimited male organ is borne terminally on a stalk of variable length arising, of course, always from a position very close to the oogonium (Fig. 7, C, c, d; G, a, b; H, c; I, i; Fig. 8, A, f, g; B, h; E; K, b).

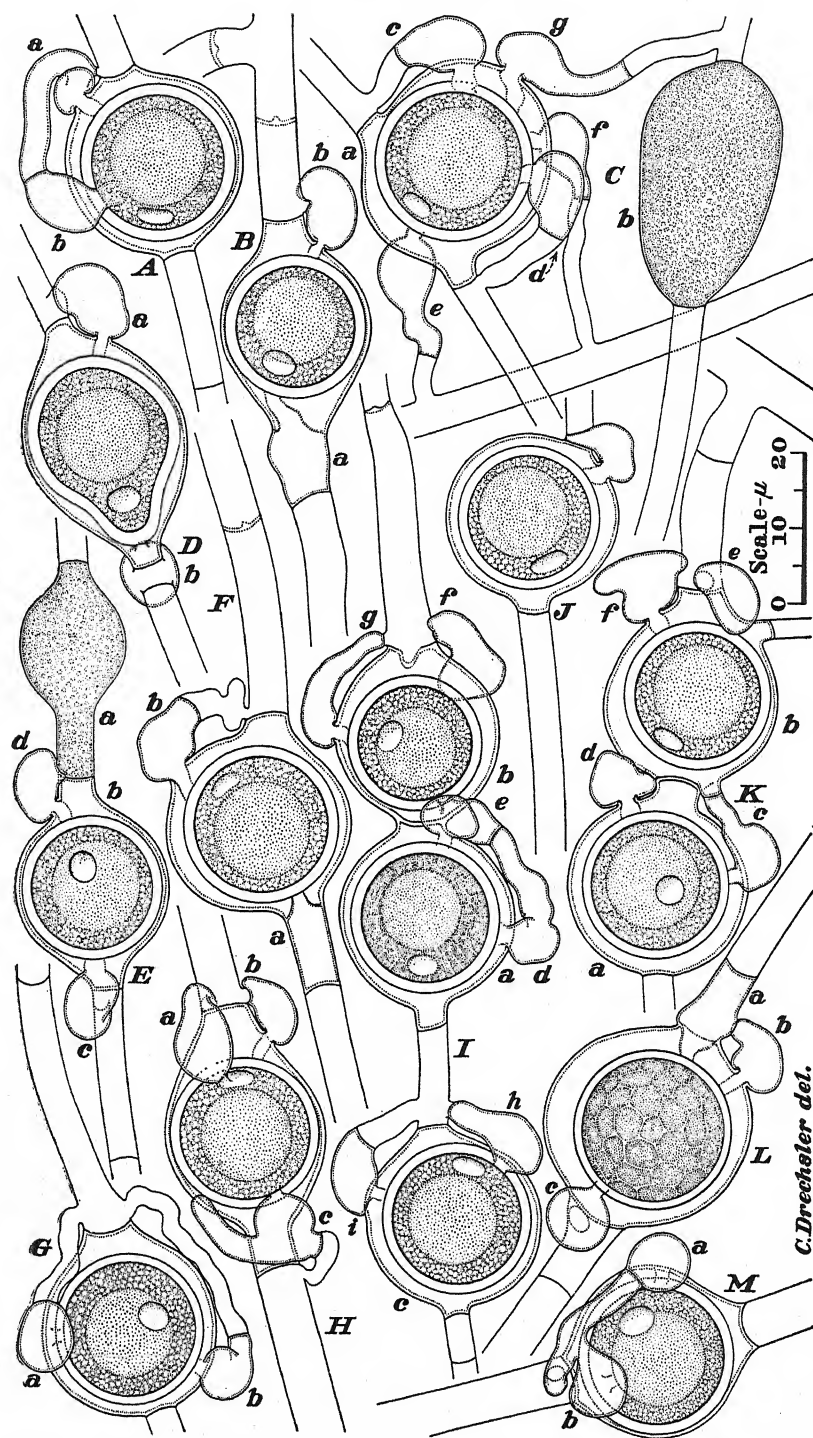


FIG. 7. Sexual reproductive apparatus of the type strain of *Pythium paroecandrum* isolated from field garlic, drawn with aid of a camera lucida; $\times 1000$ throughout.

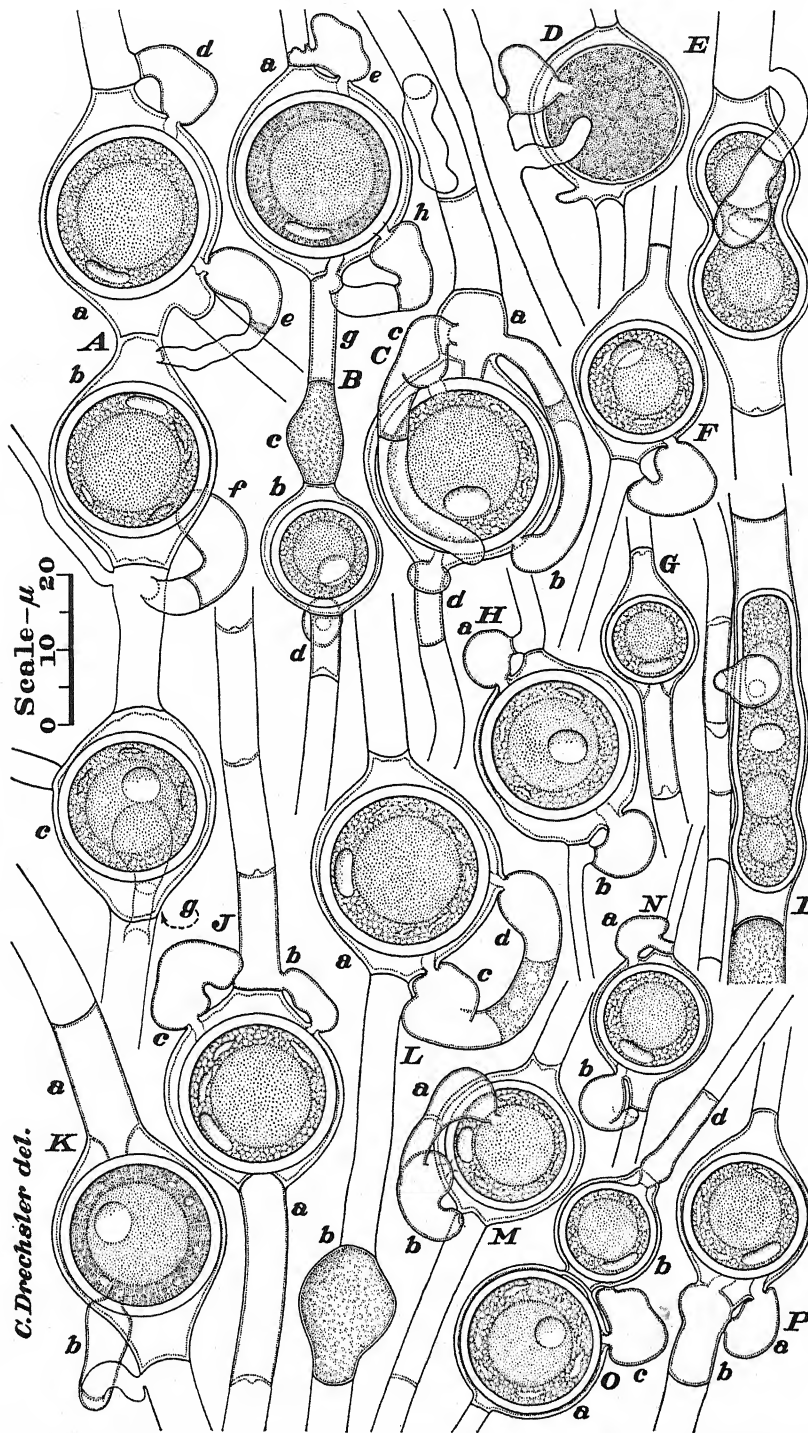


FIG. 8. Sexual reproductive apparatus of a strain of *Pythium paroecandrum* isolated from bloodroot, drawn with aid of a camera lucida; $\times 1000$ throughout.

Antheridia thus differing considerably in manner of origin are found variously associated in many units of sexual apparatus. Not infrequently a lateral outgrowth, delimited at its base by a cross-wall and divided by a median septum, comes to constitute 2 antheridia arranged in series; the basal portion, sometimes with a pouch-like excrescence of its own, serving as a male organ no less than the distal portion (Fig. 7, A, a, b; I, d, e; M, a, b; Fig. 8, M, a, b). Likewise an antheridium composed wholly or in large part of a segment of hypha adjacent to the oogonium, may be contiguous with a sessile antheridium borne laterally on it (Fig. 7, L, a, b; Fig. 8, J, b, c; P, a, b), or may lack such contiguity only because the lateral antheridium is provided with a stalk (Fig. 8, B, g, h). Occasionally 2 fertilization tubes may be intruded into an oogonium from a more or less rangy antheridial system only rather dubiously divided internally by a protoplasmic plug (Fig. 8, C, a, b; L, c, d). Now and then, too, a sessile lateral antheridium may bear distally an empty sterile hyphal prolongation (Fig. 8, C, e).

After their fertilization the oogonia of *Pythium paroecandrum* show the internal changes familiar in many congeneric species. During the earlier stages in the development of the oospore its contents appear aggregated into somewhat irregular, sizeable lumps of nearly homogeneous consistency (Fig. 7, L; Fig. 8, D). Reserve material of completely homogeneous consistency soon begins to accumulate in a globule at the center of the massed lumps. As the reserve globule increases in size the protoplasmic lumps in the narrowing peripheral layer show indications of radial orientation (Fig. 7, I, a; Fig. 8, B, a; K) before becoming resolved into finer granules. At early maturity the oospore reveals unitary structure, its relatively large single reserve globule being surrounded by a rather narrow parietal granular layer in which is embedded a single refringent body, occasionally subspherical in shape, but more usually rather strongly flattened (Fig. 7, A; B; C, a; D; E, b; F; G; H; I, b, c; K, a, b; M; Fig. 8, A, a, b; B, b; C; F; G; H; J; L, a; M; N; O, a, b; P).

In cultures showing abundant sexual reproduction 2 oogonia may often be found immediately adjoining each other, their chambers separated only by a delimiting cross-wall (Fig. 7, I, a, b; K, a, b; Fig. 8, A, a, b; O, a, b). Frequently in instances of such contiguity one of the female organs is found supplied with antheridia (Fig. 7, I, d, e; K, c, d; Fig. 8, A, e; O, c) coming from its adjacent neighbor, which, therefore, on casual inspection presents the appearance of a bisexual structure. On more careful examination the presumption of bisexuality is not sustained. Evidently the antheridia of apparently anomalous origin arise, individually, like other male organs in the species, from an unmodified portion of hypha adjoining the oogonium they are destined to fertilize, and come to have their curious positional relationship only when the portion of hypha in question is utilized directly in the production of a second oogonium contiguous with the first. Should the portion of hypha be utilized instead in the development of a contiguous sporangium, any antheridium it may have put forth will seem to have arisen from the asexual reproductive body (Fig. 7, E, d).

In *Pythium paroecandrum*, as in many related fungi, oogonia and oospores departing markedly from a spherical shape are occasionally produced. Mycelium that has become largely exhausted in reproduction seems more inclined than young mycelium to afford development of malformed oogonia, wherein may be developed cylindrical oospores measuring perhaps $40\ \mu$ in length and $10\ \mu$ in width (Fig. 8, I), or, again, oospores of shapes suggestive of a dumb-bell (Fig. 8, E). Such oospores are often extensively adnate to the oogonial wall, and internally may reveal 2 (Fig. 8, E) or 3 (Fig. 8, I) reserve globules. The partly multiplicate internal structure represented here is manifestly referable to spatial exigencies, and is therefore not to be confused with the distinctive multiplicate structure characteristic of the oospores of *P. helicoides* Drechsl. and its allies.

Oogonia and oospores of such atypical form were excluded from consideration in the 200 measurements on which were based the statements given in the diagnosis relevant to the main dimensions of the fungus. Apart from this meager discrimination the measurements were made on units of sexual apparatus selected at random in maize meal-agar cultures of the strain originating from field garlic,—each of the cultures used showing very abundant sexual reproduction with virtually no degeneration. The 200 mature oogonia gave values for diameter that when expressed to the nearest micron were distributed as follows: $11\ \mu$, 1; $15\ \mu$, 1; $16\ \mu$, 1; $17\ \mu$, 1; $18\ \mu$, 3; $19\ \mu$, 11; $20\ \mu$, 33; $21\ \mu$, 52; $22\ \mu$, 51; $23\ \mu$, 27; $24\ \mu$, 15; $25\ \mu$, 3; $27\ \mu$, 1; and the 200 oospores of correct unitary internal structure contained within the oogonia gave values for diameter that when expressed to the nearest micron showed the following distribution: $10\ \mu$, 1; $13\ \mu$, 1; $14\ \mu$, 1; $15\ \mu$, 1; $16\ \mu$, 8; $17\ \mu$, 39; $18\ \mu$, 71; $19\ \mu$, 54; $20\ \mu$, 18; $21\ \mu$, 5; $22\ \mu$, 1.

While in its asexual reproductive phase *Pythium paroecandrum* is closely similar to *P. debaryanum*, *P. irregulare* and *P. mammillatum*, it differs markedly from these species in its sexual phase; for, as has been noted, wherever its oogonia are fertilized by antheridia coming from the same filament, the antheridia in question are never borne, as usually in the 3 congeneric forms mentioned, on branches arising some distance from the female organ, but either consist in whole or in part of an adjacent portion of filament, or constitute the whole or a part of a process arising laterally from the axial filament in immediate proximity to the female organ. To be sure, the antithesis between *P. paroecandrum* and *P. debaryanum* with regard to origin of antheridia in monoclinal sexual apparatus is not a complete one, as the latter species also reveals, though only rather sparingly, male organs arising in proximate relationship to the oogonia. The same partial antithesis has been set forth earlier (6, 11) in distinguishing *P. ultimum* from *P. debaryanum*. Certainly, in their antheridial relationships, *P. paroecandrum* and *P. ultimum* present a striking parallelism. However, the mature oospore of *P. paroecandrum* differs rather markedly from that of *P. ultimum* in the greater size, proportionally, of its central reserve globule, in the correspondingly lesser thickness of its parietal granular

layer, and in the frequently much flattened shape of its refringent body. Further, of course, *P. paroecandrum* like *P. debaryanum*, *P. irregulare* and *P. mammillatum*, is separated from *P. ultimum* by its ready production of zoospores.

Before *Pythium paroecandrum* was described as new its morphological features were considered in comparison more especially with the morphological details given by Butler (3) in the original account of his *P. rostratum*. Although the measurements for diameter of zoosporangium given by Butler somewhat exceed those of my fungus, the difference could not be regarded as sufficient for the separation of 2 species. Even less disparity was evident with respect to size of oogonium. If the antheridium of *P. rostratum*, described as being generally single, and as consisting often of a short hyphal segment adjacent to the oogonium, or of such a segment together with a short lateral process arising therefrom, fails to embody the whole range of variability revealed by antheridia in monoclinal sexual apparatus of my fungus, it yet could be recognized as unmistakably embodying an important part of that range. The chief difference impelling separation was much the same as that on which Butler based the separation of his species from *P. debaryanum*. For, in *P. paroecandrum*, as in *P. debaryanum*, and also as in *P. ultimum*, during a long period assimilated to *P. debaryanum*, the oospore is considerably smaller than the oogonium, and is, therefore, loosely contained within the oogonial chamber; whereas, in *P. rostratum*, the oospore completely or nearly completely fills the oogonium. The distinction appears all the more valid from the circumstance that Butler studied his fungus in water cultures, where oospores of many species of *Pythium* tend to become smaller in proportion to the oogonium than on firm agar substrata. According to Butler, moreover, the tube of discharge in the sporangium of *P. rostratum* is characteristically thickened about midway in its length. By way of contrast the evacuation tube produced by the sporangium of *P. paroecandrum* does not show consistently any distinctive localized modification; its tendency toward occasional crookedness and toward distal widening being shared by the homologous elements of many related species.

From de Bary's publications (1, 2) on his *Pythium proliferum* and *P. ferax*, it seems clear that these fungi produce antheridia in proximate relationship to the oogonium. However, the terminal proliferous sporangium, characteristic of *P. proliferum* and found presumptively also in *P. ferax*, represents a type of asexual reproductive body differing rather widely from the more commonplace, usually intercalary, nonproliferous sporangium of the present form.

In *Pythium pulchrum*, which, according to its original description by von Minden (18), also produces antheridia adjacent to oogonia, the oogonia and oospores have average diameters of 28 μ and 24 μ respectively, and are, therefore, considerably larger than the corresponding structures of *P. paroecandrum*. The clustered basipetal development of sporangia, figured

by von Minden, has never been observed in irrigated preparation of my fungus. Several cultures that I have isolated from aquatic material and that show satisfactory agreement with the description of *P. pulchrum*, are assuredly alien to *P. paroecandrum*.

Höhnk (14), in his original account of *Pythium epigynum*, dealt with a form wherein, again, mostly intercalary, subspherical zoosporangia comparable in size to the zoosporangia of *P. paroecandrum* are associated with oogonia that only slightly exceed those of my fungus in average diameter and that apparently are regularly fertilized by 1 or 2 antheridia consisting of somewhat swollen adjacent hyphal segments. From the discussion and illustrations given by him it is not apparent that antheridia individually consisting in whole or part of a lateral outgrowth arising in immediate proximity to the oogonium were ever observed, or that fertilization tubes ever entered the oogonial chamber except by penetration of the delimiting septum. The oospores of *P. epigynum*, judging from their range in measurements of diameter, 14 to 22 μ , mostly 18 μ , appear closely similar in size to those of *P. paroecandrum*, and, incidentally, would seem to have been more plausibly described in Höhnk's arresting phrase "oogone not filling" than in the deduction he drew from an overlapping of plotted curves representing values for diameters of oogonium and oospore. The rather meager dimensional overlap suggests not so much that some oospores fill the oogonia wherein they are produced, as that some of the larger oospores produced by the species, if they could be transferred, would fill some of the smaller oogonia produced by the species, and would, indeed, more than fill other still smaller oogonia produced by it.

Matthews (17), in illustrating *Pythium pulchrum*, gives figures among which some show much resemblance to Höhnk's figures of *P. epigynum* with regard to position and origin of antheridia. If the magnifications indicated for Matthews' figures are correct, the oogonia and oospores drawn by her would appear, besides, to have had dimensions approximately equal to the homologous dimensions of *P. epigynum*, despite an implication in her account that the drawings were prepared from a culture producing oogonia even somewhat larger than the oogonia of von Minden's fairly robust fungus. However, the catenate arrangement of zoosporangia set forth by Matthews received no mention in Höhnk's account. It is not apparent that either of the 2 fungi studied by these authors had a range of variations in antheridial relationship comparable with that revealed by *P. paroecandrum*.

In describing his *Pythium piperinum* as a new species causing root rot of pan, *Piper betle* L., and of pipri, *P. longum* L., Dastur (4) mentioned that its antheridia may consist of a branch from the oogonial hypha, or may develop directly from this hypha. All 3 of Dastur's drawings of sexual apparatus show plural antheridia in positions near an attachment of the oogonium to the supporting filament, suggesting that the male organs were derived from lateral outgrowths put forth by the filament close to the oogonium. In their measurements the oogonia and oospores of *P. piperinum*

appear only slightly smaller than those of *P. paroecandrum*. However, the zoospores of the Indian fungus, measuring only 3.4 to 5.1 μ would seem not only smaller than the swarm spores of *P. paroecandrum*, but smaller than any normal swarm spores I have seen produced by any species of *Pythium*.

SUMMARY

Many zoosporangia of *Pythium dissotocum* consist of completely undifferentiated filaments, while others include a number of somewhat distended lateral branches. They yield enormous numbers of zoospores, which often are much given to iterant swarming. In the sexual apparatus of the species are revealed some antheridial relationships familiarly exemplified in *P. debaryanum* and *P. ultimum*.

Pythium periilum displays swollen elements more abundantly in its zoosporangia than *P. dissotocum*. Its oogonium is extensively and closely inwrapped by a branching antheridial filament, much like the oogonia of *P. scleroteichum* and various terrestrial species of *Aphanomyces*.

Pythium paroecandrum produces subspherical zoosporangia like those of *P. debaryanum*. As is implied in the specific epithet, a term compounded of 2 words meaning "neighbor" and "man" respectively, its antheridia often arise in close proximity to the oogonium. Thus, in arrangement of sexual apparatus, the species greatly resembles *P. ultimum*, although the oospore with its relatively large reserve globule has an internal organization more suggestive of *P. debaryanum*.

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VOLATILE FUNGICIDES, BENZOL AND RELATED COMPOUNDS, AND THE PRINCIPLES INVOLVED IN THEIR USE^{1, 2}

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INTRODUCTION

For several years the use of volatile fungicides to control downy mildew of tobacco has been given consideration both in Australia and in the United States. The information gained has been largely assembled in previous reports (1, 2, 3, 4, 6, 8, 9, 10, 13). The present paper comprises two portions. The first deals with experiments involving the application of benzol at intervals of more than one night between successive treatments, and with experimentation on types of evaporators. The second details experiments concerning the mode of action of fumigant fungicides that are basic to the subsequent discussion of the principles involved in the use of volatile compounds. This portion is timely because volatile materials, aside from benzol, are indicated as useful not only in control of tobacco downy mildew but of various other plant diseases.

APPLICATION OF BENZOL AT REGULAR INTERVALS OF MORE THAN ONE NIGHT

In our previous reports (7, 9, 13) involving the use of benzol, each successive night, as a means for the prevention and control of tobacco downy mildew in seed beds, attention was called to the fact that satisfactory control can be obtained when the interval between successive applications is longer than one night (13). The possibility of the more efficient use of materials

¹ Cooperative investigation conducted by the Virginia Agricultural Experiment Station and Duke University.

² Acknowledgment is made of the cooperation of Mr. E. G. Moss, Oxford, N. C., in the conduct of experiments at the Tobacco Experiment Station.

and labor in connection with fumigation of seed beds with benzol made it desirable to gain additional information as to the efficacy of intermittent treatments at these longer intervals. During the season of 1939, therefore, experiments involving the application of benzol regularly at longer intervals were conducted near McDonald and Oxford, North Carolina and Chatham, Virginia. These locations are representative of the area devoted to the growing of flue-cured tobacco. The applications at each of these localities were made according to a predetermined schedule. In addition, near Chatham, benzol was applied when indicated by best judgment.

The seed beds used were tightly constructed of boards. They were divided into compartments with an area of approximately 8 sq. yd., most of them having a width of 6 ft. Large beds, 10 to 12 ft. wide, were employed in a few cases. The covers were unbleached cotton sheeting of the following specifications: threads per inch, warp 64, woof 64, sq. yd. to weigh 1 pound, 2.7. The covers were thoroughly wetted after fastening them tightly in position. The surface area of the pans used for evaporating the benzol was 100 sq. in. The intervals between successive applications in the different series were 1, 3, and 4 nights, respectively. The applications were continued for approximately a month. The amounts of benzol applied were 37.5, 50, 62.5, 100 or 200 ml. per sq. yd. of seed-bed area per application. The areas involved were 40 sq. yd. in 5 compartments treated every alternate night, 382 sq. yd. in 7 compartments treated every third night, and 24 sq. yd. in 3 compartments treated every fourth night.

The outstanding conclusion from these experiments is that benzol need not be applied every night in order successfully to control downy mildew. It should be indicated that a slight amount of sporulation had taken place in all cases, prior to making the first application, and that downy mildew was completely checked in each of the series, except one, by a single application of benzol. In this case sporulation was rather abundant throughout the bed at the initiation of treatment, and benzol was applied on two successive nights to make certain that the disease was checked.

A few flecks with necrotic centers appeared on the older leaves of some of the treated seedlings. Careful daily examinations of each bed, for the occurrence of sporulation, revealed only one or two leaves bearing sporangia in any of the beds throughout the entire period of treatments. None of the seedlings in the treated beds succumbed to downy mildew, whereas all plants in nontreated control beds were attacked, and from 25 to 75 per cent of them were killed. The appearance of one such bed, part of which was treated every fourth night and the other part, nontreated, as a control, is shown in figure 1, A and B. The treated seedlings (Fig. 1, A) were essentially of sufficient size to transplant, while the few surviving seedlings in the nontreated portion (Fig. 1, B) had just begun to recover.

The Mine Safety Appliance Combustible Gas Indicator was again used (13) to measure the benzol-vapor concentration in the atmosphere of certain beds. Attention is called to the fact established in previous investigations

(13) that concentrations of benzol vapor in excess of 0.4 volume per cent are lethal to the downy mildew pathogen. The concentrations obtained during the greater portion of the nights in these beds were fungicidal with each of the amounts of benzol employed.

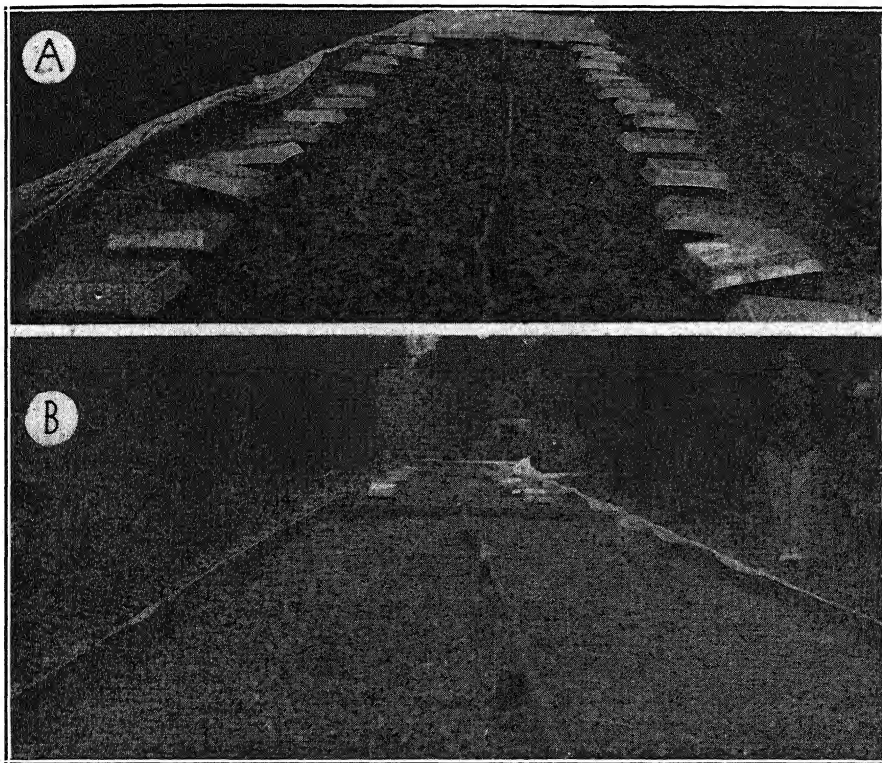


FIG. 1. A. Portion of seed bed to which benzol was applied regularly at intervals of 4 nights. (This also appears in the background of B.) B. Portion untreated. Photographs taken on same date as A.

The maintenance of fungicidal concentrations was possible by virtue of two factors, (1) moisture on the covers and foliage, and (2) amount of benzol applied. It should be emphasized that moisture on the covers and foliage, as previously stated (6, 13), is the most essential condition to the effective use of benzol in seed beds. The amount of benzol applied was in most cases in excess of what would evaporate overnight. Use of the larger amounts was followed by slight leaf-tip injury, accompanied by more or less yellowing or blanching of the older foliage and, in some cases, by dwarfing of the seedlings (Fig. 2). Under our conditions approximately 35 ml. per sq. yd. of seed-bed area appears to be the maximum quantity allowable.

APPLICATION OF BENZOL AT IRREGULAR INTERVALS

As a result of successful prevention and control of downy mildew of tobacco by applying benzol according to a fixed schedule at the longer

intervals between successive applications, it seemed logical to apply the fungicide at irregular intervals, as indicated by judgment. Time of application of benzol was based on the following factors: (1) comprehension and appreciation of influence of temperature and rainfall on severity of the disease; (2) daily observations on progress of downy mildew not only in experimental but in near-by seed beds; (3) observations made each morning on abundance of sporulation; and (4) knowledge of influence of weather on dissemination and germination of sporangia.

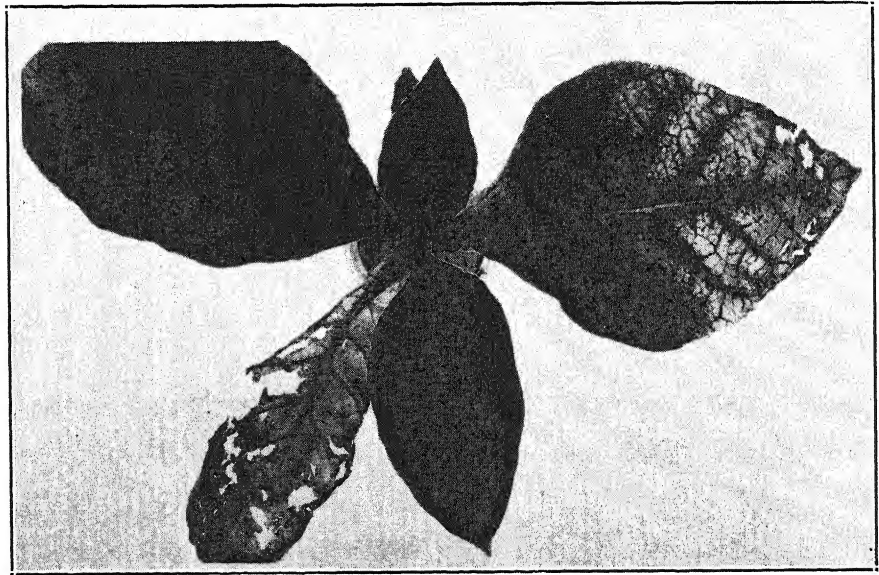


FIG. 2. Blanching of intercostal tissues characteristic of benzol injury to tobacco seedlings.

Treatment of both experimental and growers' seed beds in the manner described above was attempted near Chatham, Va. Applications of the fungicide were made first immediately after downy mildew appeared in the beds. Four to 6 additional applications so controlled the disease that the foliage was only slightly flecked.

DAYTIME APPLICATION OF BENZOL

In the hope of being able to shorten the period of treatment, several series of attempts were made to control tobacco downy mildew by applying benzol on clear warm days. Amounts ranging from 25 to 150 ml. per sq. yd. of seed bed area, were evaporated by means of pans, wick devices, and saturated compacted "cotton balls." The periods of treatment varied from 1 to 4 hours. Seed-bed covers were kept wet during the treatments. From these experiments it appears that the heavier applications result in severe seedling injury. There was evident no eradicator action from applications of 25 to 50 ml. per sq. yd., even when the period of treatment extended for

3 hours on each of 2 successive daily applications. Daytime applications promise no success because of the difficulty of keeping the covers sufficiently wet to confine the benzol vapors to the seed beds, of keeping a film of moisture on the foliage, and the ineffectiveness of short-duration treatments.

OTHER MATERIALS AND METHODS OF TREATMENT

Several preliminary tests of other volatile materials have been made to determine their value as downy-mildew fungicides. These materials included S. T. 28, prepared by The Shell Oil Co.; aniline, phenol, aniline hydrochloride, paratoluidine, and pyridine. The results with each compound, except paratoluidine, are inconclusive. Aniline and paratoluidine caused severe seedling injury, and the latter was non-fungicidal in our tests.

In a previous report it was pointed out that benzol vapor rather quickly enters into aqueous solution and that water constitutes the vehicle through which benzol becomes fungicidally active. It therefore seemed logical to test dilute aqueous solutions of benzol as sprays. These tests failed to demonstrate the practical value of sprayed benzol solutions as a downy-mildew fungicide, although their application proved beneficial. The limited fungicidal efficiency of benzol in aqueous solution derives from the large amounts of water required and the necessity of frequent applications. Its use as a spray for control of other diseases remains wholly unexplored; and the possible value of dilute solutions in soil sterilization, seems worthy of study.

Dilute aqueous solutions of aniline, aniline hydrochloride, and pyridine, sprinkled upon the seedlings, were markedly injurious, even in concentrations that gave no evidence of being fungicidal. The results with aqueous solutions of phenol indicated that this compound should be given further study as a means of controlling downy mildew.

TYPES OF EVAPORATORS

Appliances, previously used as evaporators for benzol, include pans, troughs, perforated tubes, and devices with wicks. Although all are not equally good, it is possible to secure with each type satisfactory control of tobacco downy mildew. Furthermore, it has been found that seed beds not to exceed 6 feet in width facilitate installation and care during operation of any of these types of evaporators. Also such beds obviate the necessity of trampling in them while applying the fungicide.

An ideal evaporator should be inexpensive, easy to install and replenish, and durable. It also should deflect rain and permit vaporization to proceed at a uniform rate. Efforts to devise such an evaporator have been only partly successful and have led to experimentation with "cotton balls." A cotton ball consists of 30 g. of compacted non-absorbent cotton, covered with cloth. These balls are then dipped in benzol and suspended from properly spaced stakes within the beds. One cotton ball for each 4 sq. yd. of seed bed has been found sufficient. Some canopy should be provided to deflect the rain.

GENERAL CONSIDERATIONS BEARING ON FUMIGATION WITH BENZOL

Although, in 1939, entirely satisfactory control of downy mildew was secured by application of benzol at intervals of 1 to 4 nights between successive applications in 3 widely separated localities, there remains some basis for doubt whether equally satisfactory results could be secured every season. It is probable that the disease might not yield to treatment in localities where downy mildew yearly destroys nearly all of the seedlings unless the treatments are made every night. Such appears to have been the case in the experiments of Allan, Hill, and Angell (1). They report successful control in certain tobacco-growing areas in New South Wales and Queensland, if treatments are made on alternate nights, but failure with similar treatments in other areas. From their account it is not clear that their mode of application could be considered as effective as that described here. Our results indicate that benzol acts as an eradicant fungicide, provided that (1) the proper amount of benzol be applied, (2) the rate of evaporation as modified by size of the evaporators and by temperature, be favorable, and (3) the tightness and moisture conditions of the bed favor retention of the enclosed vapors.

Since the outbreak of downy mildew in 1931, this disease was especially destructive in the United States in 1932 and 1937, and only moderately

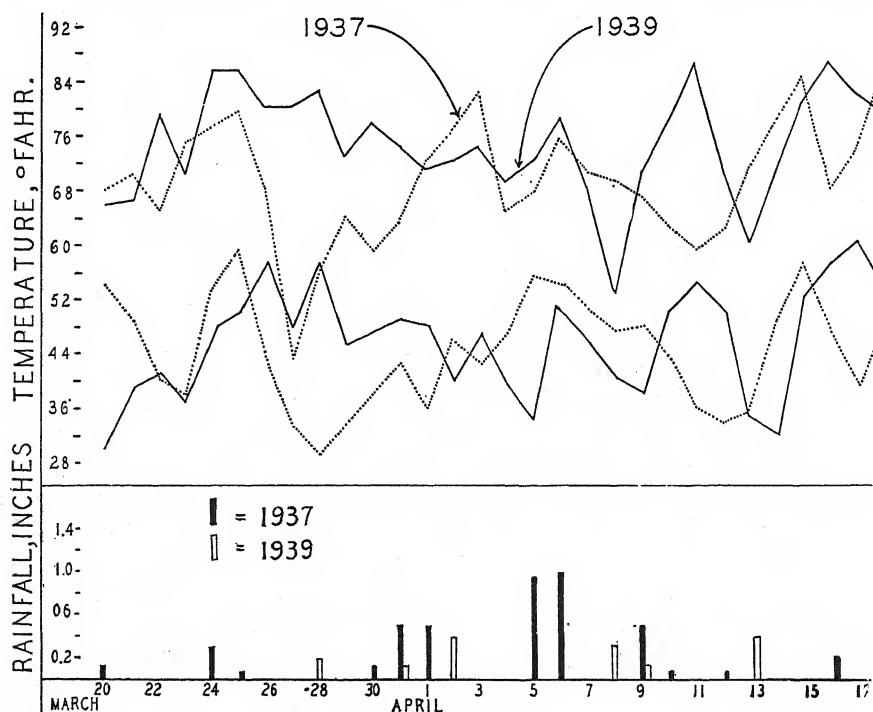


FIG. 3. Comparison of weather conditions at McDonald, N. C., from March 20 to April 16, 1937 and 1939. In 1937, 16 clear days, 12 partly cloudy or cloudy, and rainfall 4.1 inches. In 1939, 20 clear days, 8 partly cloudy or cloudy, and rainfall 1.35 inches.

severe in the remaining years. There is evidence (5) that differences in weather conditions may account for seasonal differences in destructiveness of the disease. This point is illustrated if weather conditions in 1937 and 1939, seasons in which the severity of downy mildew was strikingly different, are contrasted. Weather data for the critical 28-day periods of these two seasons, near McDonald, N. C., are presented in figure 3. It is here apparent that the season of 1937 was considerably colder than that of 1939. Almost 3 times as much rain fell in the former period as in the latter. Moreover, the proportion of cloudy and partly cloudy days was considerably greater in the former season. Whether, under such severe conditions, it is possible to control downy mildew by applying benzol at intervals greater than one night will require further field experimentation under weather conditions approximating those of the 1937 season.

EFFECT OF VOLATILE COMPOUNDS ON TISSUES OF TOBACCO SEEDLINGS AND ON
SPORES OF PATHOGENS

Experiments on the effect of volatile compounds on the tissues of tobacco seedlings and on the spores of the pathogen will be detailed before entering into a discussion of the principles that are basic to treatment with volatile fungicides. This procedure provides evidence bearing on the mechanism of operation of toxic vapors.

The Toxic Action of Benzol and Paradichlorobenzene on Tobacco Seedlings

Tobacco seedlings injured by fumigation with benzol or with paradichlorobenzene have quite the same appearance. It may be recalled that injury from benzol (Fig. 2) has been briefly described (9). Slight toxicity from paradichlorobenzene induces yellowing of the foliage and the appearance of lesions near the leaf tips. If cloudy weather follows injury of the plants, these lesions become pale brown; if clear days occur they are bleached until nearly white. This observation is based upon experiments in which portions of injured leaves were screened from direct sunlight by black paper.

In a previous report (9) consideration was given to the mode of action of benzol. It was postulated that benzol modifies the permeability of the plasma membranes by reacting especially with the lipoidal constituents of these membranes. This hypothesis was based not upon experiments designed to elucidate the mode of action but upon a rationale from the findings of those who have studied the toxicity of benzol to animals and upon the probability that the mechanism of toxic action to animal and to plant cells can be expected to accord in certain features.

In efforts to determine whether permeability of the leaf cells of tobacco seedlings is changed as the result of exposure to vapors of benzol or paradichlorobenzene, electrical conductance tests were employed. As plant physiologists well know, injured tissues exhibit increased permeability and the electrical conductivity of their fluid extracts is increased. In conse-

quence, electrical-conductance measurements have been accepted as a standard method for measuring the changed electrolyte concentration. Accordingly, leaf tissues from seedlings injured by exposure to vapors were placed in distilled water, and the samples were then put in a cold room to permit exosmosis. Tissues from normal leaves, similarly extracted, served as controls. After an appropriate interval, measurements were made by means of a conductivity apparatus equipped with audio-amplifiers and head phones. The results of representative measurements are recorded in table 1.

TABLE 1.—*Specific conductivities ($\times 10^6$, at 30°C.), expressed in reciprocal ohms, of water extract from leaves of tobacco seedlings. An interval of eight hours was allowed for exosmosis at 3°C.*

Series	Treatment of seedlings	Specific conductivity
1	Exposed over night to toxic conc. of paradichlorobenzene	404
	Nontreated control	135
2	Seedlings very severely injured by exposure to vapor of paradichlorobenzene	517
	Injured severely by treatment with benzol	1213
	Nontreated control	186
3	Exposed to vapors of paradichlorobenzene	641
	Leaves boiled in water	962
	Nontreated control	135
4	Seedlings exposed for 36 hours at 13°C. to atmosphere saturated with paradichlorobenzene	800
	Seedlings treated for 12 hours at 30°C. with paradichlorobenzene	780
	Nontreated controls	112

The most apparent conclusion deducible from these data is that leaf tissues injured by vapors of benzol or paradichlorobenzene are less able to prevent the loss by exosmosis of electrolytes. It must follow, therefore, that these volatile compounds increase the permeability of the plasma membranes. The degree of its modification apparently is directly correlated with the amount of injury or the extent to which the leaf tissues or entire seedlings collapse.

Germination of Sporangia of *Peronospora tabacina* in Solutions of Volatile Substances

A voluminous literature exists on methods of testing the fungicidal effects of chemicals. Wilcoxon and McCallan (12) state that studies on toxicity of fungicides are of 2 types: those in which quantitative measurements are made of some property of the individual, as, for example, length of germ tube or diameter of colonies; and those in which the individuals are divided into 2 categories, *i.e.*, percentage of germinated and nongerminated spores. In toxicity studies of the latter type it is well known that

each spore has its own particular lethal dose, a factor that makes impossible the procurement of a definite endpoint. Instead, it is found that a curvilinear relationship exists when the distribution of individual lethal doses is plotted against the logarithm of the concentration.

The techniques employed in tests of toxicity of non-volatile fungicidal substances have largely become standardized. This is not the case, however, at least to the same extent, with volatile fungicides, since the *sine qua non* of such tests is the use of closed germination chambers to maintain continuously the desired concentration of the chosen chemical. The chemical to be tested, moreover, should not be soluble in the material used to seal the germination chambers.

In his study of volatile fungicides Tomkins (11) used ground-glass-top jars. The molds were grown on agar solidified in the tops and inverted above the solutions of the volatile materials. Oserkowsky (8) placed vials containing saturated solutions in jars and surrounded the bases of the vials with agar. He also immersed the sclerotia of *Sclerotium rolfsii* in saturated solutions of benzol and a number of other compounds. In our experiments aqueous stock solutions, saturated at 30° C. with benzol, paradichlorobenzene, phenol, and aniline were prepared and kept in glass-stoppered bottles. Dilutions of each were made when it was desired to test fungicidal value. Hollow-ground slides, van Tieghem cells, and stoppered capillary tubes proved unsatisfactory and were discarded along with various substances used as seals. The germination chambers (Fig. 4) consisted of glass vials, 35 × 18 mm., with ground edges. These were filled almost to

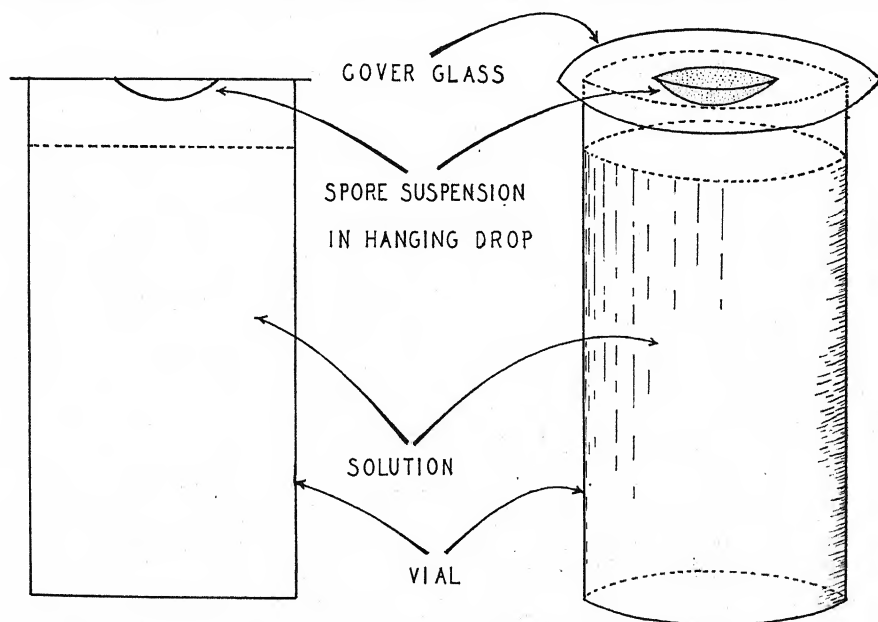


FIG. 4. Diagram of devices used in determining concentrations of volatile compounds that inhibit germination of sporangia of *Peronospora tabacina*.

the top with an appropriate dilution of the chemical to be tested. The top of each was closed with a cover glass bearing sporangia suspended in a hanging drop. A film of the test solution applied at the ground-glass edge of the vial made an effective seal, as shown by the fact that the hanging drops remained intact for 10 or more hours. Immediately after the hanging-drop suspensions had been made the germination chambers were placed in a chamber whose basal part contained water to provide them with an atmosphere of high relative humidity. Temperatures favorable for germination were then provided. The tests were started between 7 and 8 a.m. and examinations were made between 5 and 7 p.m.

Freshly formed sporangia were employed, an essential prerequisite made

TABLE 2.—*Germination of sporangia of Peronospora tabacina at 10° C. to 13° C. in aqueous solutions of benzol, paradichlorobenzene, phenol, and aniline*

Conc. of chemical	No. of tests	Results	Remarks
1/8 saturated benzol	8	No germination	Sporangia plasmolyzed
1/10 saturated benzol	3	No germination	Sporangia plasmolyzed
1/12 saturated benzol	2	No germination	Germ tubes mere protrusions
1/14 saturated benzol	6	Few sporangia germinated	Germ tubes bud-like
1/16 saturated benzol	12	In 8, few germinated; in 4, none	Germ tubes 2-4 times the length of the sporangia
1/18 saturated benzol	4	Germination abundant	Normal length of germ tubes
1/20 saturated benzol	2	Germination abundant	Normal length of germ tubes
1/24 saturated benzol	4	In 3, abundant; in 1, none	Normal length of germ tubes
1/32 saturated benzol	5	In 4, abundant; in 1, none	Normal length of germ tubes
3/4 saturated paradichlorobenzene	6	No germination	
5/8 saturated paradichlorobenzene	4	In 2, few germinated; in 2, none	Germ tubes the length of the sporangia
1/2 saturated paradichlorobenzene	12	In 8, few germinated; in 4, none	Germ tubes shorter than normal
3/8 saturated paradichlorobenzene	4	Abundant germination	Germ tubes normal length
1/4 saturated paradichlorobenzene	7	Abundant germination	Germ tubes normal length
1/600 saturated phenol	8	No germination	
1/650 saturated phenol	4	In 1, few germinated; in 3, none	Germ tubes short
1/700 saturated phenol	8	Meager amount of germination	Germ tubes short
1/750 saturated phenol	4	Many sporangia germinated	Germ tubes not of normal length
1/800 saturated phenol	2	Excellent germination	Germ tubes normal
1/50 saturated aniline	16	In 6, few germinated weakly; in 10, none	Germ tubes short
1/75 saturated aniline	12	In 6, only slight amount of germination	Germ tubes short
1/100 saturated aniline	7	Good to excellent germination	Germ tubes normal

possible by keeping a supply of infected tobacco seedlings in a box constructed to maintain temperature and moisture conditions favorable for sporulation. Control germination tests were made daily under conditions identical with that described above, except for absence of the fungicide.

A summary of the results of tests to determine the toxicity of certain volatile chemicals is contained in the following tabulation.

Apparently, concentrations of $\frac{1}{16}$ saturated benzol, $\frac{1}{2}$ saturated paradichlorobenzene, 1/750 saturated phenol, and 1/75 saturated aniline closely approximate the minimal fungicidal concentrations of these substances for *Peronospora tabacina*. It should be said in explanation that the percentage germination of sporangia was not recorded, for it is known that they vary extremely in ability to germinate in water.

Implications from Sporulation Experiments

Observations agree that sporulation may not occur on infected tobacco seedlings until 6 or 7 days after treatment with benzol, a period corresponding to the length of the sporangial cycle. It was concluded, for this reason, that benzol is lethal to the mycelium within the leaves (9). Whether it actually is killed is unknown, although this would be easily ascertainable were it possible to grow the organism in test-tube cultures. It has been im-

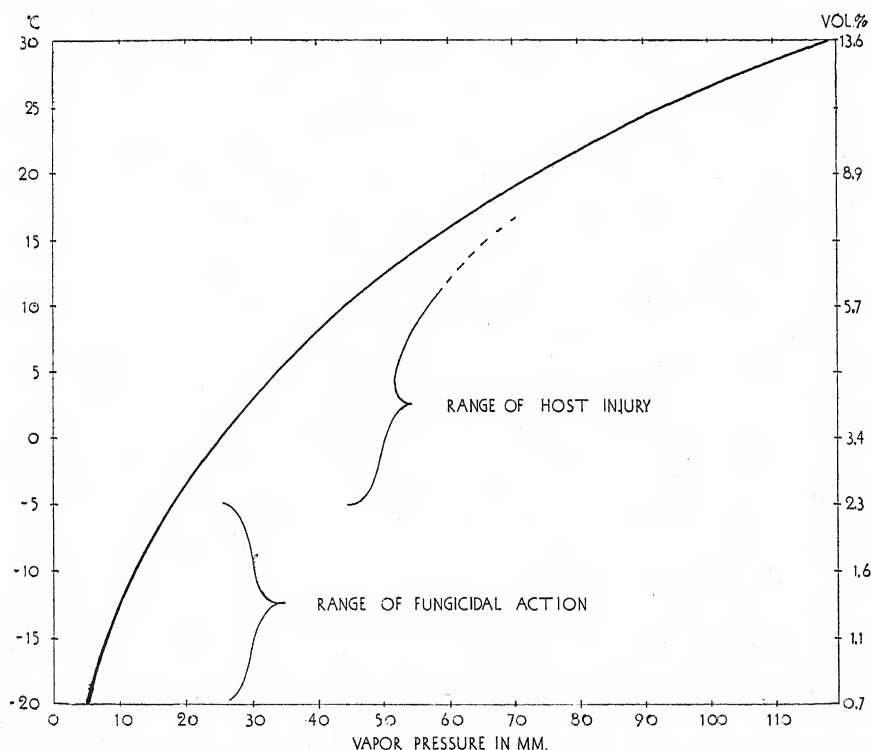


FIG. 5. Interrelationship of temperature, vapor, pressure, and volume-percentage concentration of benzol at saturation in air, showing the wide range in which benzol can be employed as a fungicide against downy mildew.

possible to determine with the microscope whether the fungus was alive or dead. No difference in appearance between the mycelium in leaves treated with benzol and that in non-treated leaves was detectable. Whether benzol produces a fungistatic or a fungicidal action is seemingly of little practical significance, since the net result is the control of the downy mildew disease. Protection may be complete if treatments are initiated prior to infection. If there is sporulation by the time of the first treatment a little flecking and necrosis may develop, but not enough to hinder transplanting.

Principles Involved in the Use of Volatile Fungicides

Studies to determine the fungicidal value of volatile materials should be based upon an understanding of certain physico-chemical principles, especially the interdependence of temperature, vapor pressure, and volume percentage concentration of the vapors in air. In order to make it possible to visualize the relation of these factors to each other in the case of benzol, and their bearing on the use of this chemical to control tobacco downy mildew, available pertinent data are presented graphically in figure 5. This curve shows the partial pressure of benzol in a benzol-saturated atmosphere at the temperature indicated. In actual field practice saturation would never be obtained. The saturation pressures are such that fungicidal concentrations are possible at temperatures much lower than those that occur while seedlings are being grown. Since fungicidal action occurs within the range 0.4 to 0.5 volume-percentage vapor concentration, it is seen from figure 5 that saturation pressures of benzol vapor below -20° C. would, in fact, be effective. However, within the temperature range for growth of tobacco seedlings effective fungicidal concentrations occur at less than 0.1 saturation. Temperature, therefore, is never a limiting factor in the effectiveness of benzol as a downy-mildew fungicide.

It may be of interest to contrast certain related volatile compounds with benzol. For example, naphthalene, closely related structurally to benzol, might be anticipated to have a very similar specific toxic action. Laboratory and field tests, however, have shown that it does not successfully control tobacco downy mildew because of its inherently low volatility. Its vapor pressure at saturation, at 10° C., is 0.021 mm., whereas the corresponding value for benzol is 45.0 mm. On the other hand, if the toxic action be altered by changing the chemical structure, certain compounds with limited volatility may become valuable fungicides. Thus, if 2 chlorine atoms are introduced in the para positions into the benzene ring, both the volatility and the specific toxic action of the aromatic nucleus are altered. Although the compound formed, paradichlorobenzene, has a saturation pressure of only 0.23 mm. at 10° C., nevertheless, it is an effective fumigant.³ Its saturation pressure (0.23 mm. at 10° C.) corresponds with a volume-percentage concentration of 0.031 per cent, being less than one-third of the volume-percentage concentration of benzol that was found necessary for

³ From our unpublished data.

fungistatic action (13). When, however, the temperature is as low as 0° C., the saturation pressure of paradichlorobenzene drops to 0.089 mm. and the volume-percentage concentration of its vapors in air to 0.012 per cent. Since actual concentrations in seed beds would be far below saturation, the magnitude of the concentrations realizable at low temperatures might be so small as to make paradichlorobenzene an ineffective fungicide.

The above figures were obtained from laboratory experiments on infected seedlings subjected to a moving air current containing a constant vapor concentration of the fumigant. Under these conditions, equilibrium distribution of the fumigant between the vapor phase and the plant tissues could be approximated.

Monochlorobenzene, structurally intermediate between benzol and paradichlorobenzene, has properties that are intermediate between the two compounds. Its vapor pressure at 10° C. is 4.9 mm., and sufficiently large concentrations of its vapors in air are obtainable to make it an effective fungicide, as has been demonstrated (7).

The mechanics of action of fumigant fungicides may be conceived to proceed as follows: When the vapors reach the leaf surface their absorption seems to take place mainly by solution in the surface film of moisture, although stomatal openings may serve the fumigant to some extent as inlets to the leaf tissues. This aqueous solution of the fumigant on the leaf surface, if sufficiently concentrated, is lethal to both sporangia and spores.

The widely differing results of treatment when the leaf surface is kept wet instead of dry during fumigation with benzol shows that solution of the fumigant plays an important part in determining its effectiveness. Although the volatile substances herein employed are commonly regarded as insoluble in water, their small solubility in water (benzol, 1.8 g./1000 g. water at 30° C., paradichlorobenzene, 0.08 g./1000 g. water at 30° C., monochlorobenzene 0.525 g./1000 g. water at 30° C.⁴) is, nevertheless, a necessary condition to their fungicidal effectiveness. Naphthalene could be expected to be ineffective because of its very low solubility in water and its low volatility.

It seems clear that these fumigant fungicides act through the medium of their aqueous solutions on and within the plant tissues, concentrations that are effective against the pathogen being lower than those that are toxic to the host. The ratio of distribution of the fumigant, between an aqueous phase and a lipoidal or waxy phase, is such as to greatly favor solubility in the non-aqueous phase. Transfer from the aqueous layers external to the cells into the lipoidal or waxy constituents of the cells occurs, even though the concentrations of volatile fungicide in the aqueous layers be small. As a result the cell constituents become profoundly modified and their permeability is broken down. Such a mechanism of action, to be effective, would require only very small amounts of the fumigant. The occurrence of such mode of action is indicated, as has been detailed, by plasmolysis of sporangia

⁴ From unpublished data obtained by H. E. Vermillion, Duke University, Durham, North Carolina.

in aqueous solutions of benzol, by direct observations with the microscope, and by increase in conductance in host-tissue extracts.

Solution of fumigant in water has direct application to its effectiveness in seed bed treatment. The volatile substance is transferred through the air from its source within the bed, and dissolves in water on the plants, on the seed bed covers, and in the soil, thus becoming widely distributed over the bed area. This moisture, furthermore, acts as a fumigant-storage medium, since water has a greater capacity, per unit volume, than air for holding the substances herein under consideration. A liter of air, for example, saturated with benzol at 10° C. contains 0.199 g. of benzol, whereas a liter of water in equilibrium with benzol-saturated air will contain 1.76 g. The fumigant reevaporates more or less uniformly over the entire seed-bed area from its solution in films of water on the surfaces within the bed, thereby maintaining a concentration of vapor over the bed long after the liquid or solid fumigant in the applicators has vaporized.

SUMMARY

In some seasons benzol need not be applied every night in order to secure control of downy mildew of tobacco or to give complete protection against this disease. The length to which the interval between successive applications can be extended with safety is probably governed by the length of the sporangial cycle and by modificatory effects of prevailing weather conditions.

Cotton balls dipped into benzol constitute effective means for vaporization of this compound in seed beds.

As the result of exposure of tobacco seedlings to vapors of benzol and paradichlorobenzene there is an increase in permeability of the plasma membranes.

Concentrations of 1/16 saturated benzol, 1/2 saturated paradichlorobenzene, 1/750 saturated phenol, and 1/75 saturated aniline closely approximate the minimal toxic limits that inhibit germination of sporangia of *Peronospora tabacina*.

The principles involved in the use of volatile fungicides are briefly discussed in relation to their possible mode of action and to seed bed practice.

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OBSERVATIONS ON TWO AMBROSIA BEETLES AND THEIR ASSOCIATED FUNGI¹

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INTRODUCTION

The symbiotic association of certain Scolytid beetles with the so-called "ambrosia" fungi has been recognized for nearly 100 years. There is, however, very little exact information about the nature of the association or about the ambrosia fungi. The beetles have been adequately described and classified by Hubbard (5), Swaine (15), and others, but the fungi have been very much neglected. Hartig, in 1844, described briefly the ambrosia fungus associated with *Xyleborus (Bostrychus) dispar* (Fabr.) and named it *Monilia candida*. Hubbard, in 1896, made an extensive study of the ambrosia beetles but included only casual observations on the associated fungi. He concluded, however, that there was more than one ambrosia fungus and that only the most closely related species of beetles cultivated the same one. The different fungi were not adequately described or differentiated by Hubbard, and no one ever has undertaken a comprehensive study of them.

During the summers of 1934, 1935, and 1936, two species of ambrosia beetles were very abundant on dying aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) in Itasca Park, Minnesota. *Trypodendron betulae* Sw. infested white birch and the aspen was infested by *T. retusum* Lec. The writers studied the life histories of the beetles and their associated fungi in nature, and the growth characteristics of the fungi in pure culture on artificial media. The work is not so extensive as would be desired and

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is by no means complete; but, since the authors will not have the opportunity of continuing the study, the data obtained are being placed on record with the hope that others may be encouraged to make more extensive studies of this interesting group of fungi.

AMBROSIA BEETLES AND AMBROSIA FUNGI

Schmidberger (11), as early as 1836, observed and described the association of beetles with the so-called "ambrosia." He observed that the beetle larvae fed upon a glistening white substance, already present in the galleries. Schmidberger did not understand the fungus nature of the substance, interpreting it as a product of the exuding sap. He applied the term "ambrosia" to it. Three years later these observations were confirmed by Ratzeburg (10), who suggested that the ambrosia was the product of a mixture of plant sap and insect spittle. The true nature of the ambrosia was first recognized in 1844 by Hartig (4), who described the fungus cultivated by *Xyleborus dispar* and named it *Monilia candida*. Although recognizing the ambrosia as a fungus, Hartig, believing in heterogenesis, concluded that it originated from the wood cells reacted upon by a substance secreted by the beetles.

For more than 60 years after Hartig's observations, the ambrosia fungi received little attention. From 1908 to 1913 Neger (6-9) and Schneider-Orelli (12) published a series of papers dealing with the subject. These investigators, working chiefly with species of *Xyloterus* and *Xyleborus*, described accurately the fungi associated with the beetles, grew some of them in artificial culture and attempted to explain the nature of the association.

According to Schneider-Orelli (12), the ambrosia fungus of *Xyleborus dispar* is transmitted to successive generations in the form of spores in the crop of the female beetle, which regurgitates them to start a culture in the new brood tunnel. Neger (9), however, thinks the spores are passed through the insect's body and survive in viable condition in pellets of excrement. Schneider-Orelli states that spores taken directly from the tunnels of *Xyleborus dispar* do not germinate, but if they are recovered from the crop of the female beetle they germinate readily.

Strohmeyer (14) has described several species of ambrosia beetles from specimens collected in the tropics. The female beetles of some of these species have special chitinous hooks or brushes on the front part of the head in which spores and mycelium of ambrosia are always found. These structures were interpreted as organs specialized for transporting the ambrosia fungus to new brood chambers.

Neger (7) expressed the opinion that the ambrosia fungus associated with *Xyleborus dispar* was an Endomycete, but neither he nor Schneider-Orelli reached a final conclusion as to the identity of the fungus. Trotter (16) recently reported the study of an ambrosia fungus associated with a species of *Xyleborus* infesting the wood of *Brownea grandiceps* Jacq. in Ceylon. He observed the "ambrosia" in material imported into Italy from

Ceylon and grew the fungus in hanging-drop cultures and observed its manner of fructification. In addition to a monilia-like growth, he observed a second layer of mycelium superimposed upon it. This second layer of mycelium formed an abundance of fusiform hyaline spores. On the basis of limited observations he concluded that the superimposed fungus was a second spore form of the first. For this supposedly pleomorphic ambrosia fungus he established a new genus, *Ambrosiomyces*, and named the type species *Ambrosiomyces zeylanicus*. The fungus differs widely from any other described ambrosia fungus.

The true ambrosia beetles belong to the family Scolytidae. They live in the sapwood, and occasionally the heartwood, of trees, infesting many different species. They are restricted mostly to weakened trees; vigorously growing trees or dead trees are rarely infested, although there are some exceptions. They bore deep into the sapwood, or even into the heartwood, each species making its own characteristic brood tunnels. The tunnels and breeding habits are of two general types. One group of genera is semi-social in habit, the beetles rearing their young in communal galleries, while in the other group each larva develops in its own separate larval chamber, excavating as it grows. The chief food of the developing larvae of both groups is the ambrosia fungus. According to Hubbard (5), the fungi associated with the beetles of the two groups may be separated into two corresponding groups on the basis of type of growth and method of spore formation.

In those genera in which the young are reared in a common gallery, the larvae have mouthparts that are not adapted to chewing wood and apparently they feed solely on the fungus. The larvae that develop in individual chambers have strong mandibles and consume wood, the fungus constituting only a part of their food. Although there is little or no experimental evidence, it usually is assumed that the ambrosia fungi, by concentrating the nitrogenous elements found in wood, provide a diet more suitable than wood alone.

The species of ambrosia beetles observed by the authors belong to the group in which the larvae are reared in individual chambers. No detailed descriptions of their life cycles or galleries have been published. The associated ambrosia fungi also have not been described in any detail, and there is no previous record of their culture on artificial media.

Trypodendron retusum Lec. This ambrosia beetle was very prevalent in dying aspen (*Populus tremuloides*) in Minnesota in 1934, 1935, and 1936, but was much less abundant in 1937 and 1938. It was not observed infesting any other species of tree. The beetles were never found infesting vigorous, rapidly growing trees or trees that had died the previous season. In nearly all cases infestation was confined to the lower half of the tree, being heaviest near the base and gradually diminishing towards the top. The upper limits of infestation usually coincided closely with the lower limit of living bark cambium. Attempts to start tunnels in living bark cambium usually are unsuccessful, the tunnels filling with sap and drowning the

beetles. Several healthy trees and infested trees were examined to determine the relation of moisture content of the wood to infestation. The water content of healthy trees averaged 65.7 per cent. That of the infested parts ranged from 70 to 80 per cent, while the upper noninfested part ranged from 60 to 70 per cent. The moisture determinations were made in June at the height of infestation. Later in the summer the trees died and the water content decreased to 50 per cent or lower, too low to permit fresh infestations. Trees with a second infestation seldom have been observed. Occasionally the beetles construct a second row of galleries beyond the first, but they have been observed only in trees with a D.B.H. of seven inches or more.

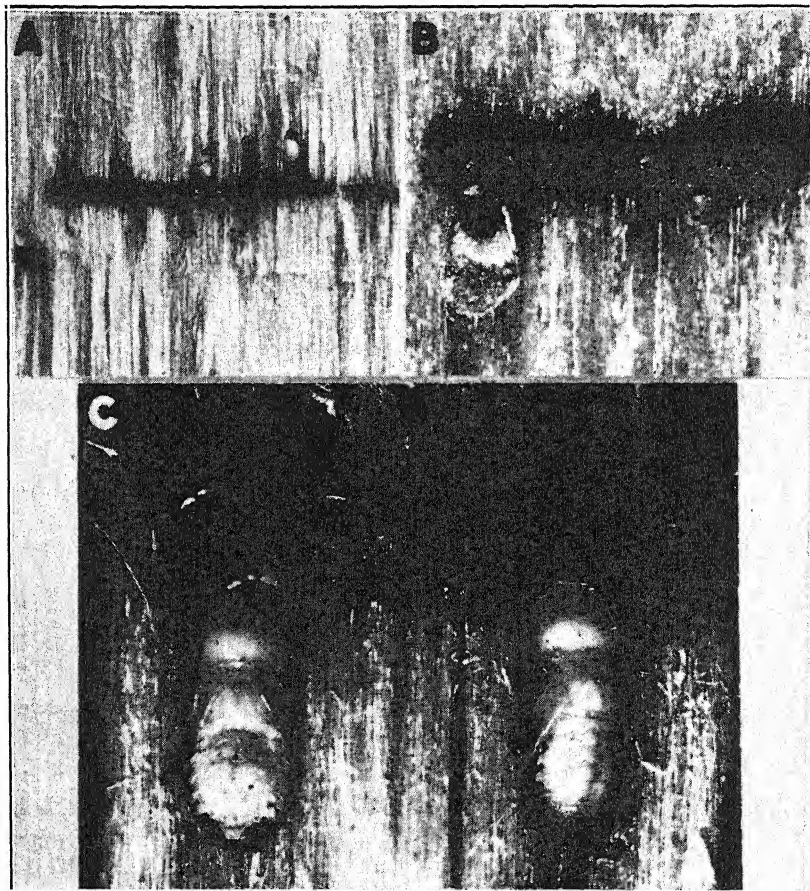


FIG. 1. Sections of brood galleries of *Trypodendron retusum* showing the larval cradles with the ambrosia fungus fruiting on the walls. A. A piece of aspen wood split through a gallery showing the vertical arrangement of the larval cradles opening off the main gallery. Two larvae are shown in place. The white ambrosia fungus may be seen fruiting on the walls of the lower cradles. Approx. $\times 1$. B. A closer view of the larval cradle showing the white masses of spores and sporophores of the ambrosia fungus. The main gallery is stained dark by the dark brown vegetative mycelium of the fungus. Approx. $\times 4$. C. Two pupae of *Trypodendron retusum* with heads toward the main gallery. Note the white masses of spores of the ambrosia fungus on the frass separating the larval cradle from the main gallery.

The overwintered beetles appear and become active in early spring and start making tunnels in the trees about the middle of May. In 1936, eggs were found in the galleries on May 23. Larvae in various stages of development were found throughout the month of June, pupation and transformation to adults occurring in early July. In late summer or early fall the new brood of adults leave the trees. They left the trees somewhat earlier in 1936 than in 1935. It was not determined where nor how the beetles survive the winter, but all available evidence indicates that they do not overwinter in the trees.

The galleries made by *Trypodendron retusum* are compound, dividing dichotomously once or twice in a horizontal plane. The single entrance tunnel extends from one-half to one-fourth inch into the wood before branching. The lateral tunnels follow a curved line parallel with the bark and usually less than one-half inch from the surface. The tunnel is excavated by the female, the male removing the accumulation of sawdust and frass. Usually 30 or more eggs are deposited in small niches at regular intervals along the roof and floor of the galleries, and each egg is covered with a small mass of sawdust and frass. The newly hatched larvae gnaw out cradles extending vertically at right angles to the main gallery, enlarging the cradles as they grow (Fig. 1, A). The sawdust and frass are forced out into the main gallery and are disposed of by the parent beetles.

Trypodendron betulae Sw. Paper birch (*Betula papyrifera*) was heavily infested with this species from 1934 to 1936, but few were found in 1937 and 1938. *T. betulae* is very similar to *T. retusum* in general appearance and life cycle, although there are a few minor differences in the construction of breeding tunnels. The tunnels of *T. betulae* extend somewhat deeper into the tree than those of *T. retusum*, usually penetrating well into the heartwood. The ambrosia fungi associated with the two beetles also were very similar, only minor differences between them having been observed. Dodge (3) reported that he had observed *T. retusum* tunneling in birch. The galleries were not completed, no eggs or larvae were found, but the fungus seemed to be able to grow satisfactorily.

The ambrosia fungi obviously are introduced into the tunnel by the adult beetles and often they may be found fruiting on the walls before the first egg has been deposited. The pads of frass covering the eggs always are permeated with the mycelium of the ambrosia fungus. On culturing the frass, the presence of contaminating fungi and bacteria can be demonstrated, but the mycelium of the ambrosia fungus always predominates. The walls of the larval cradles become overgrown with the fungus shortly after the eggs hatch (Fig. 1, A and B). The fungus layer is consumed quickly by the beetle as it enlarges its cradle, but a new crop of the fungus promptly appears. The fungus at first is glistening white, or cream colored, but the vegetative mycelium soon becomes darker. The hyaline, ovoid spores are borne in chains on short, simple sporophores arranged in a palisade layer. When a cross section of a larval chamber is examined under the microscope,

the spores and sporophores resemble the conidial stage of the powdery mildews (Fig. 2).

The mycelium penetrates the wood cells adjacent to the tunnel but rarely invades the wood for more than a few millimeters. The invading mycelium is dark-brown or black and stains the wood heavily, making the walls of the tunnels appear charred. The mycelium often fills almost the entire lumen of the cells but the walls apparently remain intact. The fungus probably obtains its nourishment from the cell contents and does not decay the cell walls. Sporulation is rare in the main galleries, and, if spores are pro-

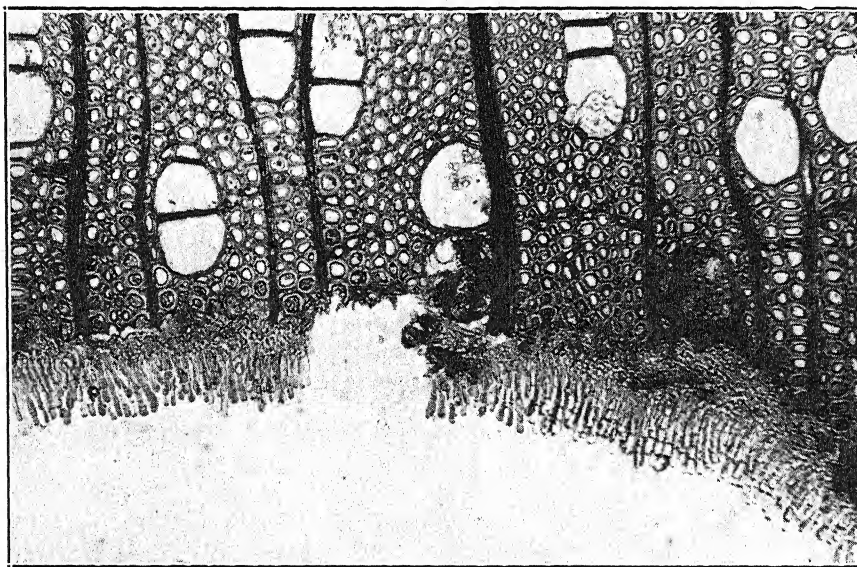


FIG. 2. Photomicrograph of cross section through a larval cradle showing the sporophores of the fungus arranged in a palisade layer on the walls of the cradle. Approx. $\times 150$.

duced, they are promptly consumed or otherwise disposed of by the adult beetles.

The fact that contaminating fungi are present has been mentioned. As long as the tunnels are inhabited by the beetles, the ambrosia fungus predominates and is not overgrown by other fungi, but if the beetles die or leave the tunnels, the contaminating fungi promptly overgrow the ambrosia fungus. It is evident that the contaminating fungi are suppressed by the beetles, but the method of suppression was not determined.

The larvae may turn around in the cradles, but are usually found facing away from the main tunnel. Immediately before pupation the larvae reverse their position and face the main gallery. The pupae occupy this position during metamorphosis, and, on maturity, the new beetle emerges by eating out the plug of frass that separates it from the main gallery and on which the fungus usually is fruiting (Fig. 1, C). The new brood of beetles evidently acquires the fungus in this way, because it was determined by

examining stained sections of the insects that none of the consumed spores or mycelium survive metamorphosis within the pupae. Pupae in various stages of development have been cultured and examined histologically, but the fungus has never been found within the body. It was not definitely determined how the fungus survives within the beetles over winter, but it is believed by the authors that spores of the fungus remain in the intestinal tract during hibernation and are voided with excrement as the beetle begins to bore the new brood tunnel in the spring.

The fungi associated with the two species are very similar in appearance

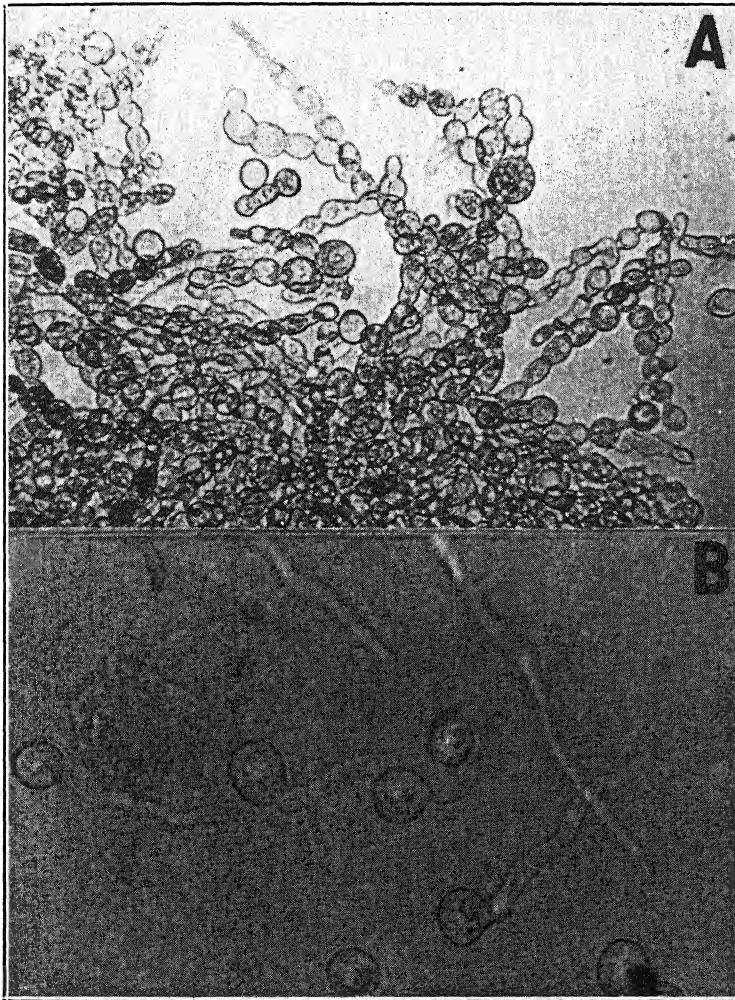


FIG. 3. A. Monilioid sporulation of the ambrosia fungus associated with *Trypodendron betulae* when first isolated in pure culture on agar. Many of the spores or spore-like cells remained attached in chains. Only a small percentage were cut off. Approx. $\times 320$. B. Germinating spores of the ambrosia fungus produced in artificial culture. Germination of spores obtained directly from the tunnels was very erratic.

and have no characters that would justify considering them as distinct species. There was considerable variation in size of the individual spores in the tunnels but the differences were not consistent enough to be significant. On germination, the spores send out a simple germ tube, but germination was very erratic, those of the fungus associated with *Trypodendron betulae* germinating more freely than those associated with *T. retusum* (Fig. 3, B). Only minor differences were observed between cultures of the fungi on artificial media. The mycelium was at first hyaline but became brown with age and the medium was discolored by a diffusible brown stain. A dark brown liquid often was exuded from the mycelium and collected in drops on the surface of the colony. Most of the studies in artificial culture were made with the fungus obtained from the tunnel of *T. betulae* because of the relative ease with which pure cultures could be obtained.

When first grown in pure culture, the fungi sporulated very poorly, forming only imperfect monilioid spores that tended to remain attached and to bud *in situ*. All gradations between distinct spores and short roundish vegetative cells were observed (Fig. 3, A). After repeated subculturing, variants that sporulated freely arose as white patches or sectors on colonies grown on agar in Petri dishes. When the patches or sectors were subcultured, cultures that sporulated abundantly and consistently were obtained, although there was considerable variation in the general appearance of the colonies formed by different sectors (Fig. 4). The spores formed by the

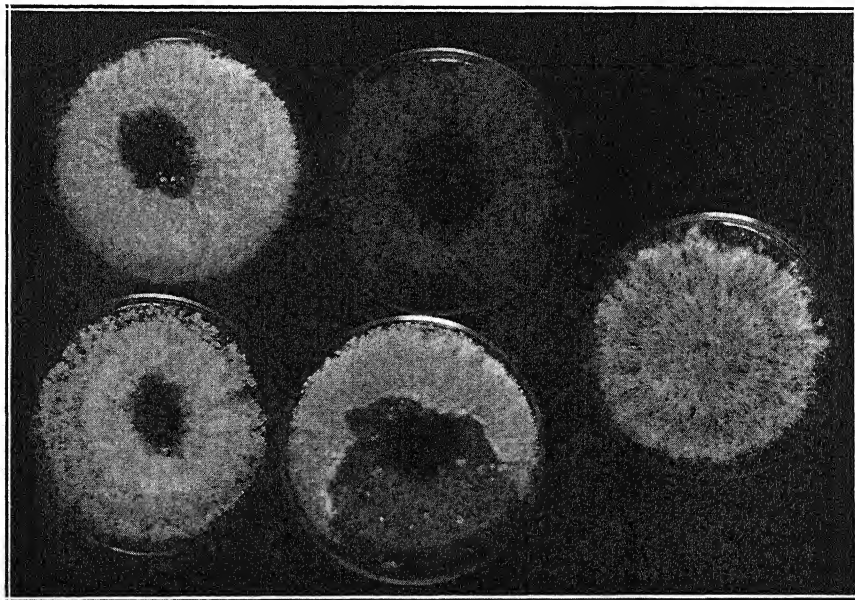


FIG. 4. Five different growth types of the ambrosia fungus associated with *Trypodendron betulae* derived from sectors obtained after repeated subculturing. The white growth consists chiefly of sporophores that produce normal subspherical hyaline spores in abundance.

variants were hyaline, with thin walls, and appeared white in mass. They were ovoid to round, tending to be more nearly spherical than the spores produced within the insect tunnels in nature. They ranged from 6 to 17 μ in length and from 6 to 14 μ in width with average dimensions of 11.38 μ by 10.09 μ . The growth of the sporophores was indeterminate and the spores were cut off basipetally.

The rate of growth of the fungus on Petri dishes of potato-dextrose agar was determined at the following temperatures: 5, 10, 15, 20, 25, 27.5, 30, and 36 degrees C. The minimum temperature for growth was about 10° C., the optimum from 25 to 27.5° C., and the maximum between 30 and 36° C. The fungus grew very well at temperatures between 15 and 30° C.

No sexual reproduction of the fungus was observed in nature or in pure culture, and, obviously, it must be classified among the imperfect fungi. Some difficulty and uncertainty are faced in referring the fungus to a genus and species. It is clearly not the same fungus described by Trotter (16) as *Ambrosiomyces zelanicus* and, in so far as the writers have been able to learn, the only other name applied to an ambrosia fungus is that applied by Hartig to the fungus associated with *Xyleborus dispar*, namely *Monilia candida*. Although Hartig's description of *Monilia candida* is not very extensive, the same fungus has been well described by Schneider-Orelli. There is enough similarity between the fungus associated with *X. dispar* and the one under consideration to justify placing them in the same genus, but they are probably specifically different. The problem is complicated still further by the confusion that has surrounded the genus *Monilia*, and especially the binomial *Monilia candida*.

Monilia was first used as a generic name by Gmelin in 1791, but was redefined by Persoon in 1801. Persoon's description of the genus is given by Stevens (13) as follows: "Hyphae erect, branched, forming a dense mycelial felt, which produces numerous conidiophores, conidia catenulate, hyaline or light-colored, ovate or lemon shaped." Castellani (2) has referred to the genus *Monilia* a number of human pathogens having quite different characters. One of these was termed *Monilia candida*, apparently without knowledge of Hartig's prior use of the binomial. Berkhout (1) made a study of the genera *Monilia*, *Oidium*, *Oospora*, and *Torula* and redefined them. The *Monilia candida* of Castellani was raised to generic rank and given the name *Candida* but unfortunately the *Monilia candida* of Hartig was overlooked or disregarded.

The ambrosia fungi associated with *Trypodendron betulae* and *Trypodendron retusum* apparently fall within the genus *Monilia* as defined by Persoon but they do not seem to be identical with *Monilia candida* Hartig. However, it is considered unwise to apply a new name to them until a relatively large number of the ambrosia fungi have been studied more thoroughly. Pending further studies they may be considered as strains of *Monilia candida* Hartig.

SUMMARY

Two ambrosia beetles (*Trypodendron retusum* Sw. and *T. betulae* Lec.), affecting aspen and birch, respectively, and their associated ambrosia fungi, were studied and described. The two fungi that were grown in pure culture are considered to be very closely related strains of one species. This species probably is not identical with any previously named fungus but, because of the lack of any extensive study of the ambrosia fungi associated with other ambrosia beetles, it was not designated as a new species.

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OVULINIA, A NEW GENERIC SEGREGATE FROM SCLEROTINIA

FREEMAN WEISS

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In two preliminary reports (5, 6) and in an article now in press,¹ a destructive disease is described that affects the flowers of cultivated azaleas in the southeastern and southern United States, and the life history of the pathogen, a hitherto undescribed fungus of the *Sclerotinia* type, is outlined. The purpose of the present paper is to present a technical description of the fungus.

¹ A flower spot disease of cultivated azaleas. U. S. Dept. Agriculture Circ. 556, 1940.

The life cycle includes the following structures or stages: (1) apothecia, the ascospores of which initiate the primary flower infections; (2) conidia, which are produced on blighted flowers and infect other flowers, giving rise to successive flower infections and conidial generations throughout the period of azalea bloom; (3) microconidia (spermatia), which are produced on infected flowers in the late stages of their collapse, and coincidentally with (4) sclerotia, which are formed in the petal tissue following its complete invasion. The sclerotia may form in infected flowers subsequent to their abscission, or in flowers that collapse and persist on the branches. Ultimately, they reach the ground, where, lightly covered with soil or remaining on the surface, they give rise to apothecia at the beginning of the host blooming period in the following, or some subsequent, year.

The flower lesions resulting from infection by ascospores are similar to those described (*loc. cit.*, p. 236) as resulting from conidial infection, but their development in nature is typically less rapid, attributable probably to the lower optimum thermal range for ascospore infection (10 to 14° C.) as compared with conidial infection (15 to 20° C.). The ascospores germinate typically with 1, sometimes 2, polar germ tubes. Penetration of the cuticle of the azalea petal by the germ tubes is preceded by blanching of the petal color in a minute halo about the point of entrance and causes conspicuous tearing of the cuticle, indicative of forceful entrance (Fig. 3, D). The mycelium grows rapidly between the cells, causing rapid loss of coherence. The hyphae branch extensively, and the main strands attain a diameter of 8 to 12 μ . They are distantly septate (100 to 250 μ) and densely protoplasmic in the juvenile portions. Ultimately the host cells become prevailingly free and suffer loss of color and structure, while the fungus permeates the fluid matrix derived from their disorganization. The petal tissue may retain a semblance of structure, chiefly through the support of the mycelium by the vascular elements and the cuticle, but when one pinches an infected petal lightly between the fingers, the tissue collapses and exudes sap. The expansion of a single lesion may be limited to 1 petal, but multiple infections typically result in invasion and collapse of the entire corolla.

Conidial production then begins, predominantly in the thin and delicate limbs of the corolla, and only to a limited extent in the corolla tube. Numerous short branches arise on the hyphae that complete the invasion of the host tissues; these become bifurcate and ultimately Y- or T-shape (Fig. 2, A, 1 and 4). The free ends enlarge, becoming globose or ovoid, and a septum forms across the branch just below the enlargement (Fig. 2, A, 2). A second septum then divides the apical portion into a small basal cell and a large conidium (Fig. 2, A, 3). The latter enlarges to its mature dimensions, while the basal cell ceases to grow and ultimately serves only as a disjuncter (Fig. 2, A, 3 and 4). By growth of the conidium and by extension of the branch that bears it, the conidia penetrate the cuticle, which becomes torn and shredded (Fig. 2, B). As the spore-bearing branches are produced in close proximity over the entire surface of the lesion, a mat or palisade of

conidia is formed on the surface of the petal (Fig. 1, B). They have been observed to number as many as 225 spores per sq. mm. of petal surface. Conidial production predominates on the upper (inner) surface of the petal, but may occur on the outer surface also, or anywhere on the corolla. Only 1 conidium is borne by each branch. Once the conidia are free above the surface of the petal, they are very readily detached from the conidiophores, bearing the disjuncter cell with them, so that they normally appear very unequally bicellular. As the disjuncter cell is empty, and often ruptures at or following abscission, the conidia are properly regarded as unicellular with a basal appendage.

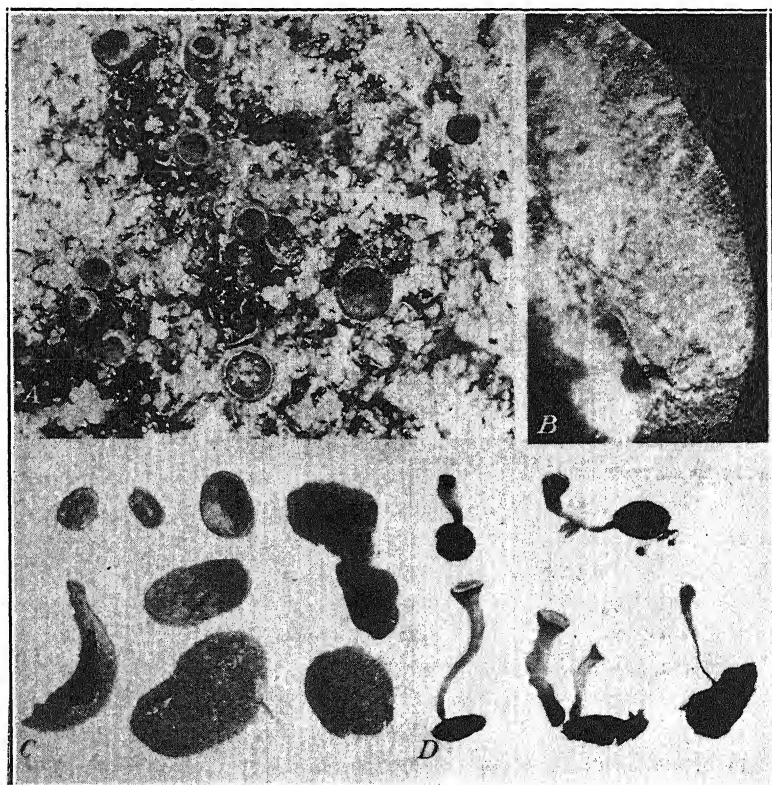


FIG. 1. A. Apothecia of *Ovulinia azaleae* emerging from sclerotia buried in sand. $\times 2$. B. Portion of azalea petal showing layer of conidia on or just above its surface, originating from subcuticular mycelium. $\times 5$. C. Sclerotia, showing cupulate form, smooth inner surface and verrucose outer surface. $\times 5$. D. Apothecia, showing occasional multiple stipes. $\times 2$.

The conidia are ellipsoid to ovoid or obovoid, with the base and apex equally convergent, or either may be larger and the other smaller (Fig. 3, C, 1). Sometimes (apparently associated with abnormally moist conditions) the conidia become greatly elongated, with a broadly clavate or pyriform shape (Fig. 3, C, 2). The conidia germinate promptly upon contact

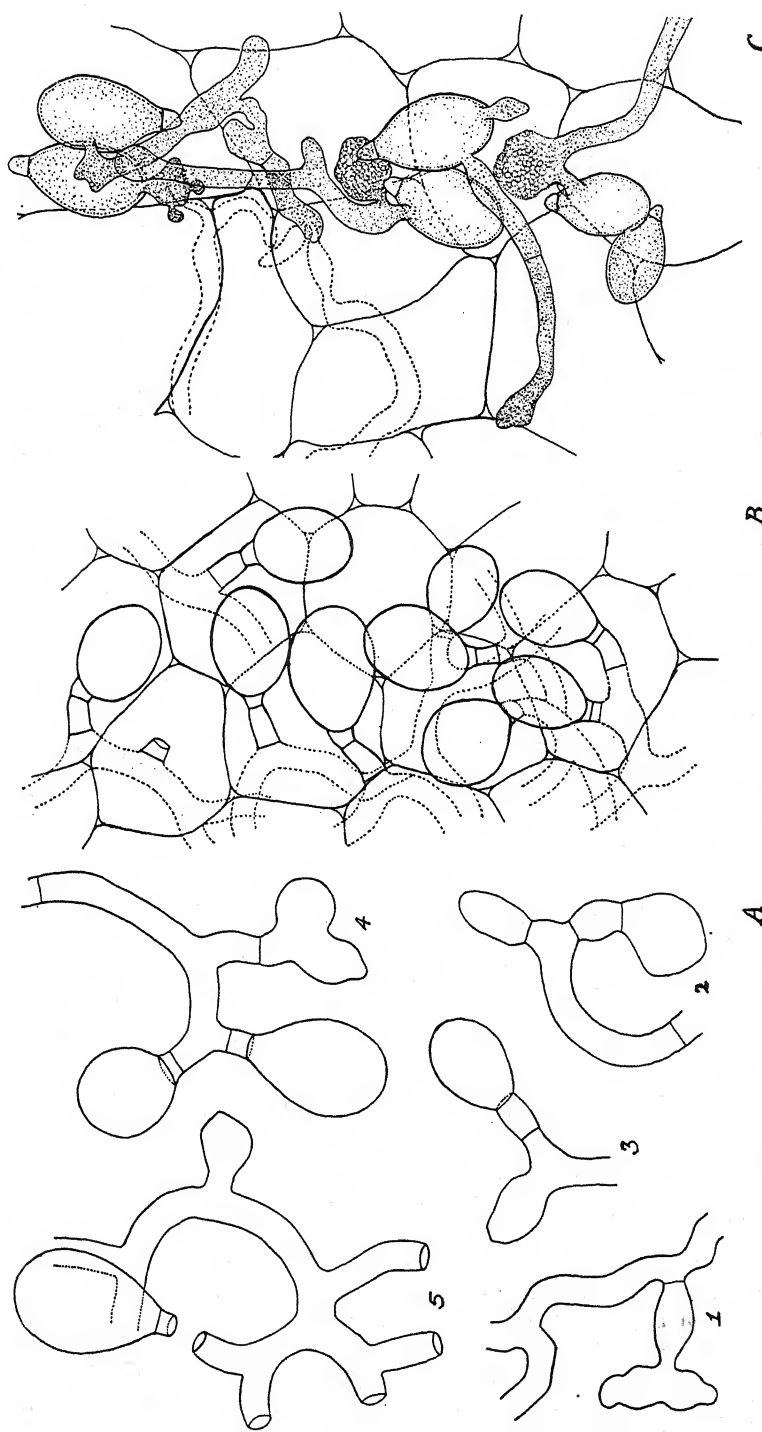


FIG. 2. A. Stages in development of conidia of *Onulima azaleae* on subcuticular mycelium in azalea petal. B. Group of mature conidia lying on surface of petal, showing conidiophores and vegetative hyphae. C. Germination of conidia and penetration of superficial cells of azalea petal by the germ tubes. $\times 275$.

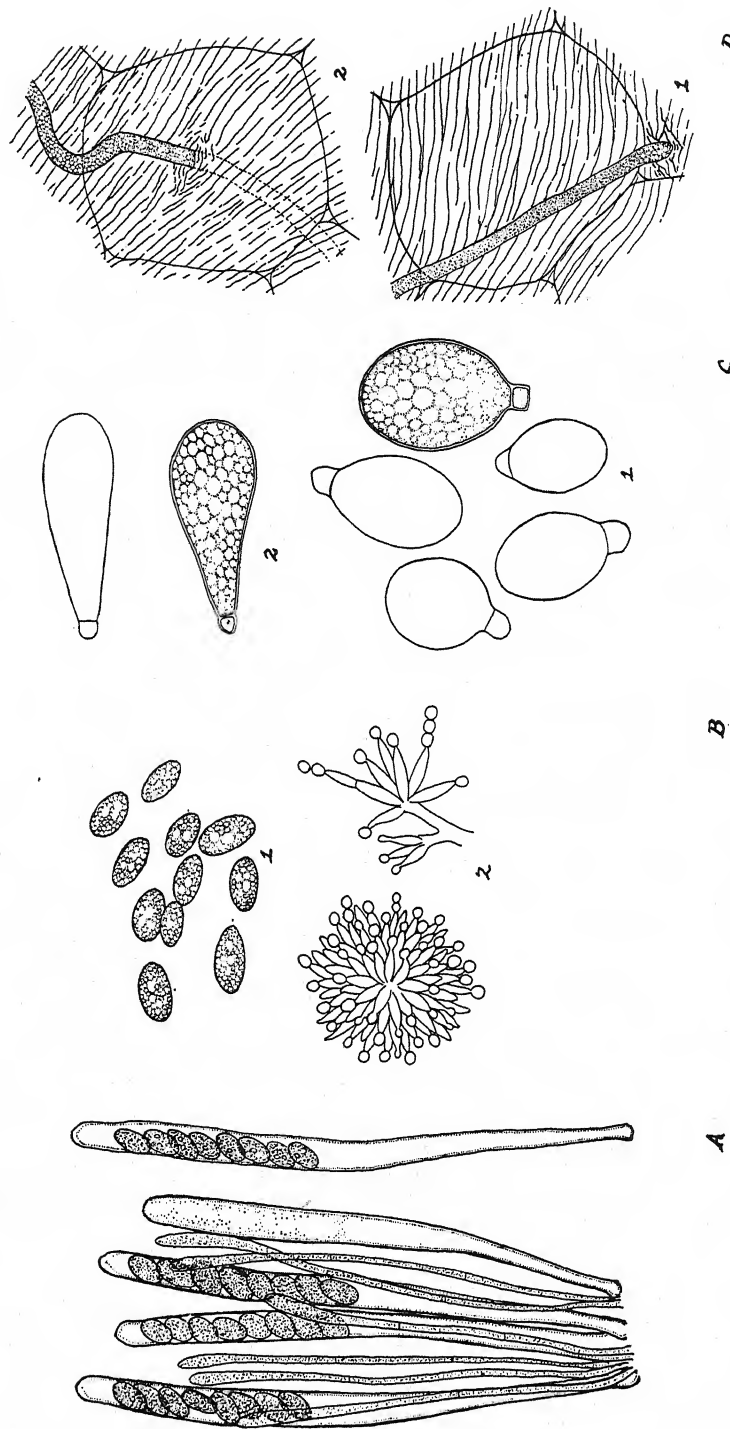


FIG. 3. A. Group of asci and paraphyses. $\times 400$. B1. Ascospores. B2. spermatidochium and portion of same showing origin of spermatia. $\times 475$. C. Normal conidia and elongated forms produced under excessive moisture. $\times 400$. D. Penetration of striate cuticle of azalea petal by ascospore germ tube, showing buckling of germ tube, rupture of cuticle, and pore left after passage. $\times 400$.

with water, usually with 1 germ tube opposite the basal cell, but often with multiple germ tubes from lateral positions. They often germinate in place in such numbers as to produce a macroscopically visible weft (Fig. 2, C). When kept dry, they may remain viable for 4 to 6 weeks at temperatures prevailing in nature during the period of the disease, and 8 to 12 months (rarely over 1 year) in artificial storage at 5 to 10° C. Their viability is not destroyed by severe freezing (−18° C.) under these conditions.

The so-called microconidia, actually spermatia (7) are produced in chains from cushions of short, fusoid hyphae (spermatophores), the whole constituting a spermadochium, on the surface of the blighted flower (Fig. 3, B, 2). They are formed contemporaneously with the sclerotia.

The sclerotia first appear as blister-like swellings within the collapsed flower tissue; they are typically more numerous and larger in the thick tubular portion of the corolla. They are at first translucent and gradually darken and become black; they vary in shape from disciform to irregular, sometimes consisting of 2 or more portions joined by a narrow isthmus, and are distinctly cupped (Fig. 1, C). The convex side corresponds to the outer surface of the corolla. The concave side is smooth, and the convex side verrucose to rugose. From 1 to 20, commonly 2 to 5, sclerotia develop in a single flower. There is a distinct cortex and a medulla. The sclerotia are formed within the host tissue but are separate therefrom and appear to contain only fungus elements.

During the final stages of development of the sclerotia, they exude drops of a clear amber fluid. Minute spines are present on the margin of the sclerotium and distributed over the convex surface. One might suppose that the spines are receptive organs, the spermatia the male elements in a sexual cycle, and that the drops of fluid serve some purpose in promoting the fusion of the two, but no definite evidence of this nature has been obtained. Neither is it known whether the fungus is heterothallic, as definitely monosporous ascospore infections have not been obtained. However, mononidial infections may give rise to both sclerotia and spermatia.

The sclerotia may produce apothecia after storage on slightly moistened peat and sand for 9 to 10 months or may remain dormant for 1 to 2 years and then give rise to normal apothecia. The temperature and other requirements for apothecial production have not been precisely ascertained, but there has been no consistent difference resulting from storage of the sclerotia at different temperatures between 5 and 18° C., though the actual production of apothecia has not occurred until the temperature was raised to 10–14 degrees. Freezing the mature, dry sclerotia (−18° C.) for several months did not destroy their vitality.

The fungus grows slowly on artificial media. On agar + sugar media it showed a preference for dextrose and a reaction approximating pH 6; but, on these substrates, it produces only a tough mat of grayish-white to buff mycelium, occasionally with sclerotium-like knots, which have not been induced to develop further. On vegetable media, such as steamed wheat or

barley, and on bean plugs, it produces sclerotia of more typical form and spermatia in abundance. These sclerotia have even borne spines similar to those formed in nature, but they have not yet been induced to produce apothecia. Conidial production has not been obtained in artificial cultures, but only on flowers following inoculation with ascospores, conidia, or mycelium.

DISCUSSION

The characteristics of the sclerotium and the apothecium obviously relate the fungus to *Sclerotinia*. In pathogenesis also it shows a marked similarity to the *Vaccinium*-infecting *Sclerotinia* spp. described by Woronin (8), Woronin and Nawaschin (9), and Fischer (1). None of these species is reported on azaleas, however, and they are described as developing only in the ovary (berry or capsule, depending on the host species) of infected flowers. Their conidial stages are all of the *Monilia* type.

In manner of conidium production the azalea fungus differs from any form as yet connected with *Sclerotinia* (4). Honey (2, 3) listed the macroconidial stages of *Sclerotinia* as belonging to the following types:

1. Macroconidia in chains
 - a. Disjunctors present
 - b. Disjunctors absent
2. Botrytis-like
3. Penicillium-like

His genus *Monilia* (2) was erected to include the species of *Sclerotinia* having macroconidia in chains. The only additional genus since segregated from the *Ciborioideae* (subfamily including *Ciboria* and *Sclerotinia*) is Whetzel's *Septotinia* (7), which differs widely from any of the above in its septate conidia and branched conidiophores.

There being no generic type in this grouping that produces conidia singly on simple conidiophores, the writer, following the lead of Honey and of Whetzel, proposes the erection of a new genus, for which Whetzel has suggested the name *Ovulinia*, with the type species *O. azaleae*.

It may be noted here that a fungus referable to *Botrytis cinerea* very commonly occurs on azalea flowers that have been damaged by abrasion, frost, or beating rain. It forms sclerotia not only in the petal tissue, similarly to *Ovulinia*, but also in the calyx, capsule, filaments, and style, where the sclerotia of *Ovulinia* have not been found. The globose or thick sclerotia of *Botrytis* are readily distinguishable from those of *Ovulinia*, which are always flat or cupulate. The writer also has collected an apothecium of the *Ciboria* type, arising from a pseudosclerotium formed within the anthers of cultivated azaleas, which had overwintered on the ground. This apothecium differs distinctly in macroscopic and microscopic characters from the apothecium of *Ovulinia azaleae* and a separate description will be given.

OVULINIA, N. GEN.

Apothecia *Sclerotiniae* similis, e sclerotiiis orientia; asci tenues, cylindrici, inoperculati; ascosporae 8, ellipsoideae, unicellulares, hyalines, uniseriatae; paraphyses ple-

rumque simplices, teretes, apicibus inflatae; conidia magna, obovoidea, unicellularia, appendicula basali praedita, hyalina, solitaria ex apicibus ramulorum brevium simplicium nata; spermata (microconidia) minuta, globosa, in catenulas in hyphis brevibus fusoides caespites formantibus nata; sclerotia disciformia vel irregularia, tenuia, leniter cupulata, nigra.

Apothecia of the *Sclerotinia* type, arising singly or in groups from sclerotia. Asci slender, cylindrical, inoperculate; ascospores ellipsoid, 1-celled, hyaline, typically 8 in 1 series; paraphyses mostly simple, terete, tips swollen.

Conidia large, obovoid, 1-celled, with a basal appendage consisting of a sterile disjunct cell, hyaline, produced singly at the tips of short, simple branches from a parasitic mycelium within, and forming a mat on the surface of the host organ.

Spermata (microconidia) minute, globose, produced in chains on short fusoid hyphae forming tufts (spermadochia) on the surface of the host, accompanying the formation of sclerotia.

Sclerotia disciform to irregular, thin, shallowly cupulate, black, formed within but discrete from the host tissues.

OVULINIA AZALEAE²

Apotheciis solitariis vel 2-3-caespitosis ex quoque sclerotio, stipitatis, urceolatis vel cyathiformibus, dein appianatis, 2-5 mm. latis, fulvo-olivaceis usque brunneis, margine scabroso, granuloso vel hirsuto; stipite 2-3 mm. (interdum 15-18 mm.) longo, 1-1.5 mm. crasso, recto vel leniter curvato, ad basim argillaceo, apicem versus cinnamomeo, fere glabro; hymenio brunneo, pruinoso; ascis cylindricis, 140-260 μ longis, 9-14 μ crassis; ascosporis 8, uniseriatis, ellipsoideis, unicellularibus, 10-18 \times 8.5-10 μ , hyalinis, 1-3-guttulatis; paraphysibus teretibus, septatis, plerumque non ramosis, apice leniter inflatis; conidiis obovoideis, hyalinis, appendicula basali inclusa 40-60 \times 21-36 μ , solitariis ex apicibus ramorum brevium simplicium natis, de conidiophoris e cellula disjunctori conidio affixa manerenti separantibus; spermatis globosis, 3.0-3.5 μ diam., apicibus hypharum fusoidarum 10-12 μ longarum, 3 μ crassarum, in caespites aggregatarum orientibus; sclerotii disciformibus vel irregularibus, cupulatis, nigris, 2-5 \times 3-10 \times 0.5-1.5 mm. Parasitica in floribus *Rhododendri* spp. e Carolina superiore usque Texas, U. S. A., etiam flores *Rhododendri*, *Kalmiae*, et *Vaccinii* spp. artificiose inficiens.

Apothecia arising singly or in groups of 2 to 3 (rarely up to 8) from the margin of a sclerotium (Fig. 1, A, D), lying on or shallowly covered with soil, in late winter and early spring, stipitate, urceolate to cyathiform, flat at maturity, 2-5 mm. broad, tawny olive to snuff brown, margin scaly, granulose or hirsute; stipe typically 2-3 mm. long, 1-1.5 mm. thick, erect or slightly curved, but sometimes sinuous, filiform, and up to 15-18 mm. long, clay-color (R) at the base, darkening to cinnamon at the top, glabrous, rarely with 1 or a few rhizoids; hymenial surface russet to walnut brown, somewhat pruinose. Asci cylindrical, 140-260 μ (average 180 μ) long by 9-14 μ (average 12 μ) thick; apical plug not staining blue with iodine (Fig. 3, A). Ascospores 8, uniseriate, ellipsoid, 1-celled, 10-18 \times 8.5-10 μ (average 16.3 \times 9.3 μ), hyaline, usually with 1-3 prominent globules (Fig. 3, B, 1). Paraphyses terete, septate, mostly unbranched, apices slightly swollen.

Conidia typically obovoid, hyaline, 40-60 \times 21-36 μ (average 50 \times 28) including the basal appendage; when formed under high humidity becoming clavate to pyriform, up to 72 μ long; produced singly on short simple branches protruding from the host surface and arising from a parasitic mycelium underneath; separating from the conidiophores by means of a disjunct cell which remains attached to the conidium (Fig. 2, A; 3, C). This stage forms a thin mat or web on the surface of the host organ and the conidia are promptly disseminated therefrom by insects and meteoric water or germinate in place.

Spermata globose, 3.0-3.5 μ in diameter, produced at the tips of fusoid hyphae, 10-12 \times 3 μ , which are aggregated into minute tufts (just visible by a 10 \times lens) on the host surface (Fig. 3, B, 2); usually separating readily but sometimes adhering in short chains; appearing coincidentally with sclerotia.

Sclerotia formed within invaded host tissues but separable therefrom when mature; typically of circular to elliptical outline, often irregular, distinctly cupped, smooth on the concave surface, verrucose to rugose on the convex (Fig. 1, C); 2-5 \times 3-10 \times 0.5-1.5 mm.; cortex and medulla differentiated structurally but at maturity black throughout.

Growing readily on 2 per cent potato-dextrose agar at pH 6.0 when recently isolated, forming a coarse, tough, mat-like mycelium, grayish white to pale fawn in color, becoming stromatoid and dark in color with age and tending to be short-lived. On vegetable media (bean pods, barley or wheat kernels) sclerotia and spermata are also formed, but no macroconidia. Optimum temperature for growth 18 to 22° C.

² I am indebted to Miss Edith K. Cash for the preparation of the Latin diagnosis.

The ascospores and conidia infect flowers of cultivated azaleas and rhododendrons (*Rhododendron mucronatum*, *R. pulchrum*, *R. simsii*, *R. obtusum*, and *R. catawbiense*) causing a destructive flower blight in the southern and southeastern United States from North Carolina to Texas; pathogenic experimentally to a wider host range, including the flowers of native azaleas of the eastern United States, *Kalmia* and *Vaccinium*.

Herbarium material: The type specimens are deposited in the Mycological Collections of the Bureau of Plant Industry, United States Department of Agriculture, at Washington, D. C., under Nos. 71105-71108. Duplicate material is deposited in the Plant Pathology Herbarium at Cornell University, Ithaca, New York.

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INVASION OF SWEET-CORN PLANTS OF DIFFERENT AGES BY STRAINS OF PHYTOMONAS STEWARTI

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(Accepted for publication October 16, 1939)

The sweet-corn wilt bacterium, *Phytomonas stewarti* (E. F. S.) Bergey *et al.*, is usually more destructive on young seedlings than on older plants. It produces large leaf lesions, causes diffuse wilting, and usually kills the seedlings, as described by Ivanoff (2). Older plants may be extensively invaded but are less severely injured. Seedlings are very susceptible, but some of those that are not killed outright recover, continue growth, and become sufficiently resistant with age to produce mature plants, as observed by Wellhausen (9). This observation suggests that either the morphology or physiology of maize changes sufficiently to alter the host-parasite relationship as the plant grows beyond the seedling stage, or the bacteria are modified in old plants.

It was considered of interest to test the virulence of several strains of *Phytomonas stewarti* for plants of different ages. The strains selected for these studies were B-11, the single-colony isolates obtained from it and numbered B-1011, B-1211, and B-1111, and several single-colony isolates obtained from strain B-1111. The origin, virulence for young seedlings, and physiological characteristics of B-11, B-1011, B-1211, and B-1111 have been

TABLE 1.—*Invasiveness of 10 strains of Phytomonas stewarti in sweet-corn plants inoculated at different ages*

Strain tested	Age of test plants	First test				Second test				Average infection index in 2 tests
		Condition of leaves			Infection index	Condition of leaves			Infection index	
		Total no.	No. dead	No. of lesions		Total no.	No. dead	No. of lesions		
	<i>Days</i>									
B-1011	7	59	5	41	.95	55	8	54	1.42	1.18
	14	70	1	66	.99	71	5	60	1.06	1.02
	19	83	0	72	.87	86	0	88	1.02	.95
	24	110	0	118	1.07	103	0	83	.81	.94
B-11	7	59	6	41	1.00	61	2	46	.85	.93
	14	68	1	58	.90	67	2	61	1.00	.95
	19	84	0	72	.86	85	0	70	.82	.84
	24	101	0	93	.92	99	0	95	.96	.94
B-1211	7	62	0	0	.00	64	0	1	.01	.01
	14	76	0	4	.05	75	0	7	.09	.07
	19	88	0	12	.14	86	0	16	.19	.16
	24	105	0	29	.28	98	0	19	.20	.24
B-1111	7	66	0	2	.03	60	0	4	.07	.05
	14	75	0	7	.09	72	0	9	.12	.11
	19	83	0	31	.37	85	0	30	.35	.36
	24	103	0	28	.27	103	0	87	.85	.56
B-1111-8	7	65	0	0	.00	61	0	2	.03	.02
	14	72	0	5	.07	77	0	4	.05	.06
	19	85	0	10	.12	89	0	15	.17	.14
	24	96	0	47	.49	103	0	50	.49	.49
B-1111-11	7	66	0	1	.01	63	0	5	.08	.05
	14	75	0	6	.08	72	0	7	.10	.09
	19	84	0	18	.21	85	0	16	.19	.20
	24	103	0	35	.34	98	0	35	.36	.35
B-1111-3	7	65	0	7	.11	63	0	1	.01	.06
	14	76	0	14	.18	75	0	2	.03	.11
	19	86	0	17	.20	82	0	13	.16	.18
	24	101	0	45	.45	97	0	25	.26	.35
B-1111-5	7	63	0	5	.08	58	0	6	.10	.09
	14	73	0	14	.19	73	0	6	.08	.14
	19	86	0	16	.19	87	0	17	.20	.19
	24	100	0	18	.18	99	0	13	.13	.15
B-1111-6	7	62	0	13	.21	65	0	9	.14	.17
	14	71	0	18	.25	72	0	9	.12	.19
	19	83	0	10	.12	85	0	21	.25	.19
	24	107	0	58	.54	100	0	36	.36	.45
B-1111-14	7	60	0	39	.65	52	1	27	.58	.62
	14	74	0	35	.47	71	0	29	.41	.44
	19	85	0	39	.46	87	0	53	.61	.53
	24	103	0	28	.27	95	0	34	.36	.31

described elsewhere (5, 6). The single-colony isolates obtained from B-1111 were similar to the parent culture except for differences in virulence. At the time the present tests for virulence were made, strains B-11 and B-1011 were capable of using inorganic nitrogen very readily; B-1111-14 used it sparingly, and the remaining cultures used it with difficulty, if at all. In other words, the weakly virulent cultures employed in these tests differed from the virulent ones in that they required organic nitrogen for their growth.

A nutrient-dextrose broth subculture of each strain was inoculated into 7-, 14-, 19-, and 24-day-old sweet-corn plants of the variety Golden Bantam. Duplicate groups of 10 plants each in the 4 age groups were inoculated with each strain. Each plant was injected at the crown and at 2 points along the leaf whorl. The plants were observed for infection 13 days after inoculation and records were taken for the calculation of an infection index based upon the average number of necrotic lesions produced per leaf (5). The data obtained and presented in table 1 show that, although there were some minor variations, the duplicate tests agreed very closely.

The highly virulent strain B-1011 was very invasive on plants of all age groups. Although the averaged data for the 2 tests show that the culture produced fewer lesions on the older plants, the differences are not statistically significant. The virulent strain B-11 and moderately virulent B-1111-14 gave similar results. On the other hand, the weakly virulent

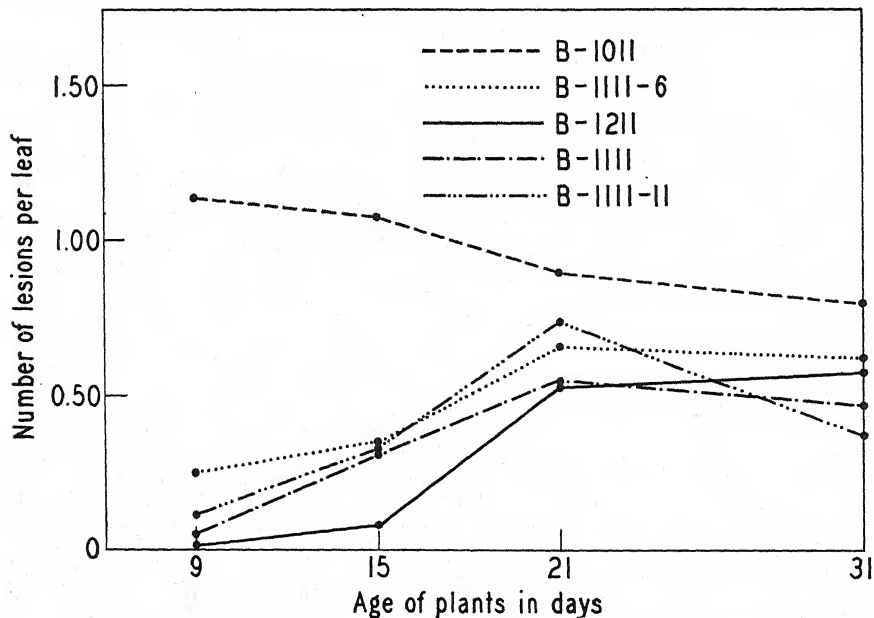


FIG. 1. Average number of necrotic lesions produced by different strains of *Phytonomonas stewartii* on leaves of sweet-corn plants of different ages. There was a tendency for strain B-1011 to produce fewer lesions on the leaves of older plants even though it did cause a general wilting. The strains that were almost avirulent for young seedlings caused distinct lesions in plants over 15 days old.

strains were, without exception, more invasive in the 2 older groups of plants. They produced necrotic and chlorotic streaks on leaves of the 19- and 24-day-old plants, even though they usually failed to cause visible lesions on 7-day plants. The lesions produced ordinarily were not so large as those caused by the more virulent strains, and, consequently, the plants were not so severely injured.

A repetition of this test using B-1111-14, B-1111-11, B-1111-6, B-1111-3, B-1111, B-1211, B-1011, and B-11 on 9-, 15-, 21-, 31-, and 57-day-old plants gave comparable results. The data obtained on B-1011, B-1211, B-1111, B-1111-6, and B-1111-11 are presented graphically in figure 1. The weakly virulent strains produced more lesions on the older plants than on the younger ones. The data on the 57-day-old group are not presented in figure 1 because these plants, which were in tassel at the time of inoculation, did not show distinct lesions. However, the plants were invaded extensively by both the weakly virulent and highly virulent strains and showed considerable general wilting. The most severely invaded leaves from representative plants of the 9-, 21-, and 31-day-old groups are illustrated in figure 2.

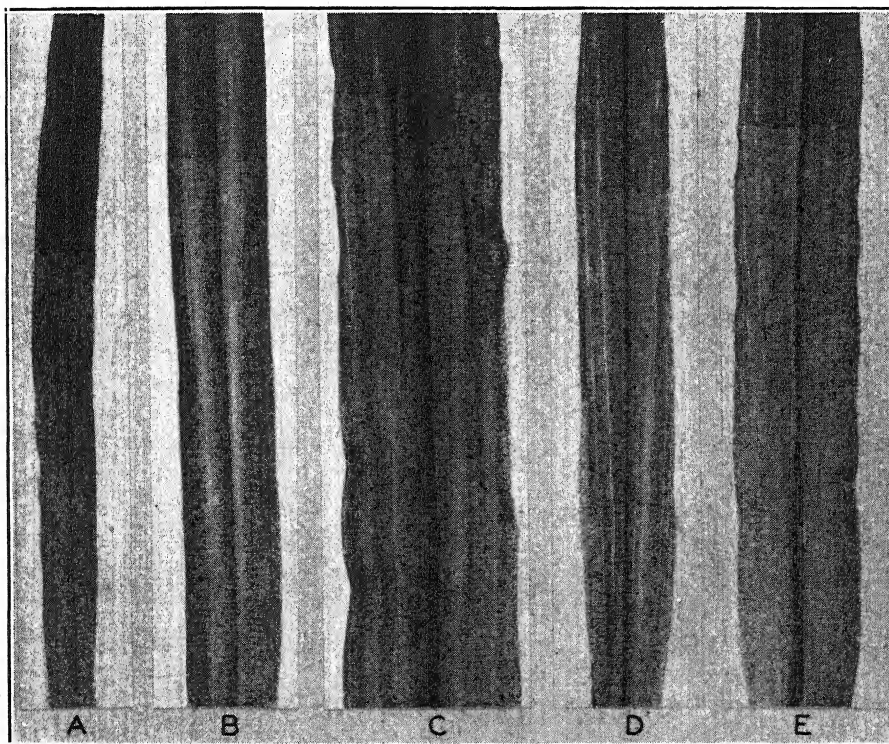


FIG. 2. Leaves from sweet-corn plants inoculated with strain B-1211 when the plants were 9 (A), 21 (B), and 31 (C) days old and with B-1011 when they were 21 (D) and 31 (E) days old. Each leaf was the most severely invaded one from a typical plant in each age group. (Photograph by J. A. Carlile.)

In order to determine whether the virulence of the strains was changed by incubation in the host, bacteria were recovered from leaf lesions and stems of several plants in each age group and tested for virulence on 10-day-old seedlings. The data obtained confirmed those reported previously (6) and may be summarized as follows. Cultures B-11 and B-1011 were not definitely affected but some of the isolates from plants inoculated with B-1111-14 were more virulent than the strain injected. Isolates obtained from the necrotic lesions on 19- and 24-day-old plants inoculated with strains B-1111 and B-1211 were not appreciably more virulent than the parent cultures. However, virulent isolates were observed in 1 of the 6 groups tested. Some of the bacteria recovered from the stems of 24-day-old plants severely invaded by B-1111 were almost as virulent as B-11. These virulent derivatives from B-1111 were found to be capable of utilizing inorganic nitrogen when transferred to the synthetic medium used for such tests (6).

DISCUSSION

The data presented in this paper show that the relationship of host and parasite changes as sweet-corn plants grow older. The highly virulent strains produce no more lesions on old plants than on young ones, but the weakly virulent strains become distinctly more invasive as the plant grows beyond the seedling stage. The obvious conclusion is that there is some change in the host's physiology that affects the weak strains' ability to multiply. The nature of this change is not immediately obvious, but, as mentioned before, it may be assumed that the change affected either the host's susceptibility or the bacteria's virulence. The latter possibility can be dismissed because weakly virulent strains were recovered from the lesions they had produced. The fact that only weakly virulent strains were isolated from some plants argues against the idea that the bacteria had been changed before they invaded. The virulent isolates obtained from some plants inoculated with weakly virulent strains apparently developed at random in the host, just as they do in cultures (6) of the weakly virulent strains.

There is no direct evidence of a change in the growing plant that would permit B-1211 and B-1111 to become invasive as the plant ages. Since it has been shown (6) that the impotency of these strains is caused by their inability to grow on inorganic nitrogen, a logical assumption to explain the observations reported above would be that organic nitrogen appears in the tracheal tubes as the plant passes from the seedling to the independent stage. There is not enough known about translocation of organic nitrogen in maize to verify this assumption. It has been shown that practically all of the reserve proteins in the seed are converted into soluble compounds (3) and are transported to the growing tips of the plumule and root (7, 8), where they accumulate as asparagine or combined proteins (7) within the first week after the seed begins to germinate. Protein nitrogen from the seed, therefore, does not enter into the present discussion because the period of infection was 2 to 4 weeks after germination. Any organic nitrogen that

reaches the xylem during this later period must be synthesized by the plant from carbohydrates and inorganic nitrogen. Such a synthesis might very well be delayed until after the second week of growth when the plant had produced sufficient leaf area to manufacture carbohydrates. If so, the change in host metabolism must have occurred at the same time the host became more susceptible to strains such as B-1211. The organic nitrogen produced by the growing plant may have passed from the phloem into the adjacent xylem tissue. At least carbohydrates reach the xylem in sufficient quantity to support bacterial growth. It is also known that organic nitrogen occurs in the tracheal sap of other plants (1, 4).

SUMMARY

Virulent strains of *Phytomonas stewarti* were as invasive on young sweet-corn seedlings as on more mature plants. Weakly virulent strains, on the other hand, were much more invasive on plants that were over 14 days old than on younger plants. Since these weakly virulent strains were obligate users of organic nitrogen, the hypothesis is advanced that organic nitrogenous compounds appear in the tracheal tubes after the plant has become established and has started synthesizing its own organic materials.

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PREVALENCE OF CUCUMBER AND TULIP VIRUSES IN LILIES

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Until recently, lily mosaic was believed to be caused by a single virus, peculiar to lily and of very limited host range. In 1937, McWhorter (3) demonstrated that a virus of the tulip group occurs commonly in symptomless lilies of certain species, and Price (5) isolated a strain of Cucumis Virus I from Easter lily (*Lilium longiflorum* Thunb.) showing typical necrotic fleck symptoms. The writer (1, 2) has presented evidence that the viruses described by McWhorter and by Price are distinct, and that viruses of both these classes can be recovered from the typical necrotic fleck type (Fig. 1, B) in Easter lily. However, the strong mottle in Easter lily (Fig. 1, A) has yielded only a tulip virus. In commercial forcing of Easter lilies only fleck symptoms are commonly interpreted as mosaic, since plants carrying strong mottle, or carrying only McWhorter's latent virus, are satisfactory for forcing.

McWhorter demonstrated the existence of his latent virus of lily by inoculating the juice of lilies into healthy tulips by hypodermic needle. We have compared this tulip test with that of rubbing young leaves of *Lilium formosanum* Stapf. Parallel trials of 23 individual Easter lily plants by these two methods are compared in table 1. Symptoms in *L. formosanum*

TABLE 1.—Comparative results of inoculating Clara Butt tulips and *Lilium formosanum* seedlings as index plants for tulip virus in Easter lilies

Source plant			Results of inoculating	
Number	Description	Symptoms	Tulips	<i>L. formosanum</i>
C ₁ 37-1	Creole	None	^a 4/6	^a 4/4
C ₂ 37-1	do	do	3/6	3/4
F 23-1	do	do	0/6	4/5
C ₁ 37-39	do	Mottle	4/6	10/10
C ₁ 37-17	do	Fleck	1/6	3/4
C ₁ 37-113	do	do	4/6	4/4
Ct 36-33	Croft	None	5/6	1/4
Ct 37-16	do	do	1/6	2/4
Ct 37-24	do	do	0/6	3/4
Ct 37-74	do	do	0/6	0/4
E ₁ 37-241	Erabu	do	2/6	4/4
E ₂ 37-26	do	do	4/6	3/4
E ₂ 37-48	do	do	0/6	2/4
G 37-48	Giganteum	do	1/6	3/4
G 37-63	do	do	2/6	4/4
H 37-136	Harrisi	do	3/6	4/4
H 37-123	do	Mild mottle	4/6	4/4
WQ 7	White Queen	None (seedling)	0/6	0/4
WQ 142	do	do	0/6	0/4
152-2C-1	Seedling	None	0/6	0/4
152-2T-9	do	do	0/6	0/4
152-3C-7	do	do	0/6	0/4
GS 85-7	do	do	0/6	0/4

^a Number of plants infected over number inoculated.

were recognizable in 10 to 14 days after inoculation; those in tulips were expressed in the year following inoculation. It appears from these data that *L. formosanum* is a satisfactory index plant for tulip virus in lilies. The agreement between tulip and *L. formosanum* as test plants is reasonably close with a few discrepancies suggesting that the lily test is more efficient

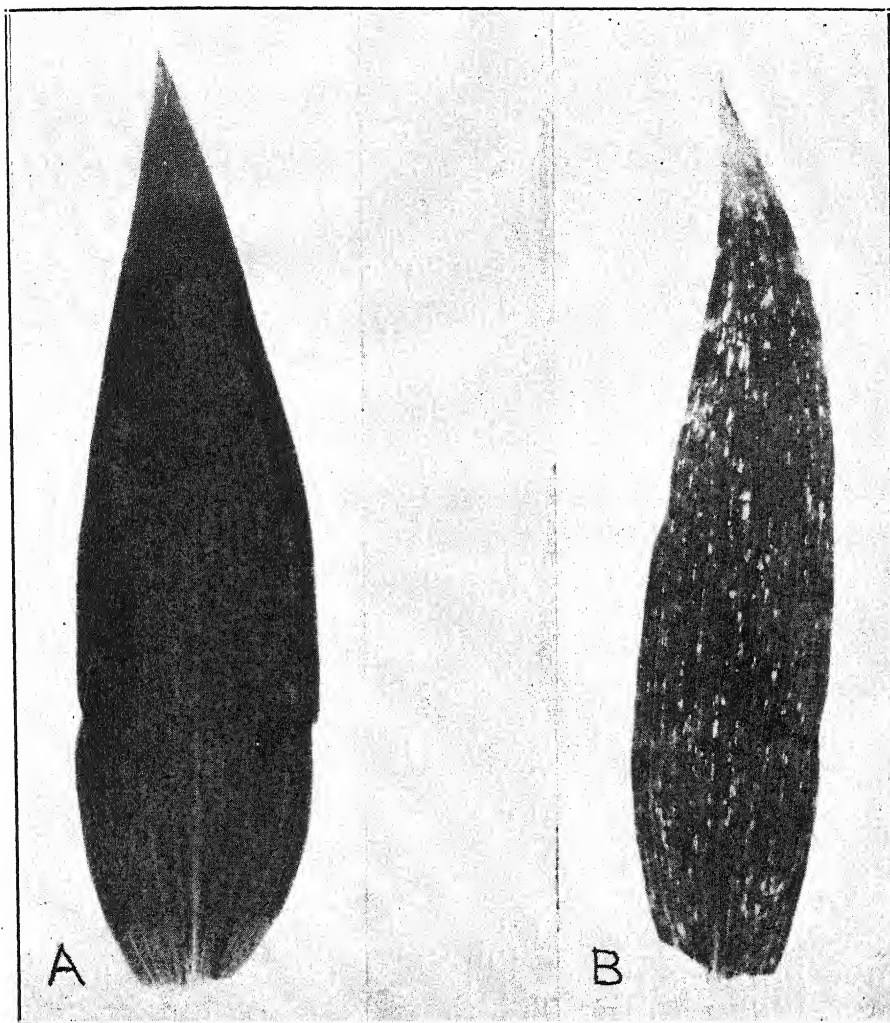


FIG. 1. Mosaic symptoms in *Lilium longiflorum* var. Creole. A. Strong mottle. B. Typical necrotic fleck.

than the tulip test, as well as much faster. It is not yet known whether these discrepancies represent merely differences in the efficiency of the methods in our hands or a differential susceptibility of the test plants.

The Easter lilies tested in the trials listed in table 1 include symptomless selections from Louisiana Creole, Florida Creole, Oregon Croft, Japa-

nese Erabu and Giganteum, and Bermuda Harrisii. The individual source plants were selected during the forcing of 200 to 600 of each commercial variety, as plants free from virus symptoms throughout the forcing period. The fact that all but one of these symptomless lilies showed evidence of latent tulip virus strongly suggests that this virus is present in nearly all commercial Easter lilies from all sources. At the same time 6 green-house-grown seedlings tested virus-free on both tulip and *Lilium formosanum*.

Price (5) used Turkish tobacco as a test plant in demonstrating cucumber virus in lilies. We have adopted this test and, combining it with the *Lilium formosanum* test, have used a simple mechanical inoculation of these 2 species as an index of the presence of cucumber and tulip viruses in lilies. A representative set of plants for such indexing is shown in figure 2, A, and another set is shown in figure 2, B, 9 days after inoculation from a specimen of the hybrid lily George C. Creelman, which proved a carrier of both tulip and cucumber viruses. Carborundum dust was applied to the leaves rubbed in all inoculations. Turkish tobacco is inoculated while very small, as shown in figure 2, A. *L. formosanum* responds satisfactorily at any stage of active growth when new leaves are developing. We have regularly washed off the inoculum shortly after the operation is finished, since the juice of lilies will often burn tobacco leaves if allowed to dry in place.

Infection in Turkish tobacco is considered evidence that a cucumber virus is present. Symptoms usually appear as white necrotic rings in 4 to 6 days, and may or may not be followed by systemic mottling. If no infection appears in tobacco, but *Lilium formosanum* develops yellowing and curling of the young leaves in 6 to 10 days, followed by mottling of various types, a tulip virus is considered present. If both test species respond, as shown in figure 2, B, the plant indexed carries both viruses. During hot summer weather *L. formosanum* may develop a mild mottling in young leaves from cucumber mosaic alone, but this can be distinguished from tulip virus on subsequent development.

It has not yet been proved that all viruses isolated from lily and that produce symptoms in Turkish tobacco belong to Cucumis Virus I, but it seems probable that they will be so classed. There is less reason for regarding as strains of a single virus all collections that are positive in *Lilium formosanum* and negative in tobacco, but this working hypothesis is maintained for the present. The possibility remains that other viruses not detected by this index test occur in lily, but the conception of 2 distinct viruses, each including a number of strains, seems adequate to account for most of the virus patterns thus far encountered in our study. The yellow flat or rosette type (4) has not been recognized in our material.

During the summer of 1939, garden lilies of many species and varieties from a number of localities were indexed by the method described above. Material was collected by the writer and by S. L. Emsweller, or mailed to this station by E. P. Imle and others. Five plants of each test species were used in each index trial. In 56 representative trials in which tulip virus

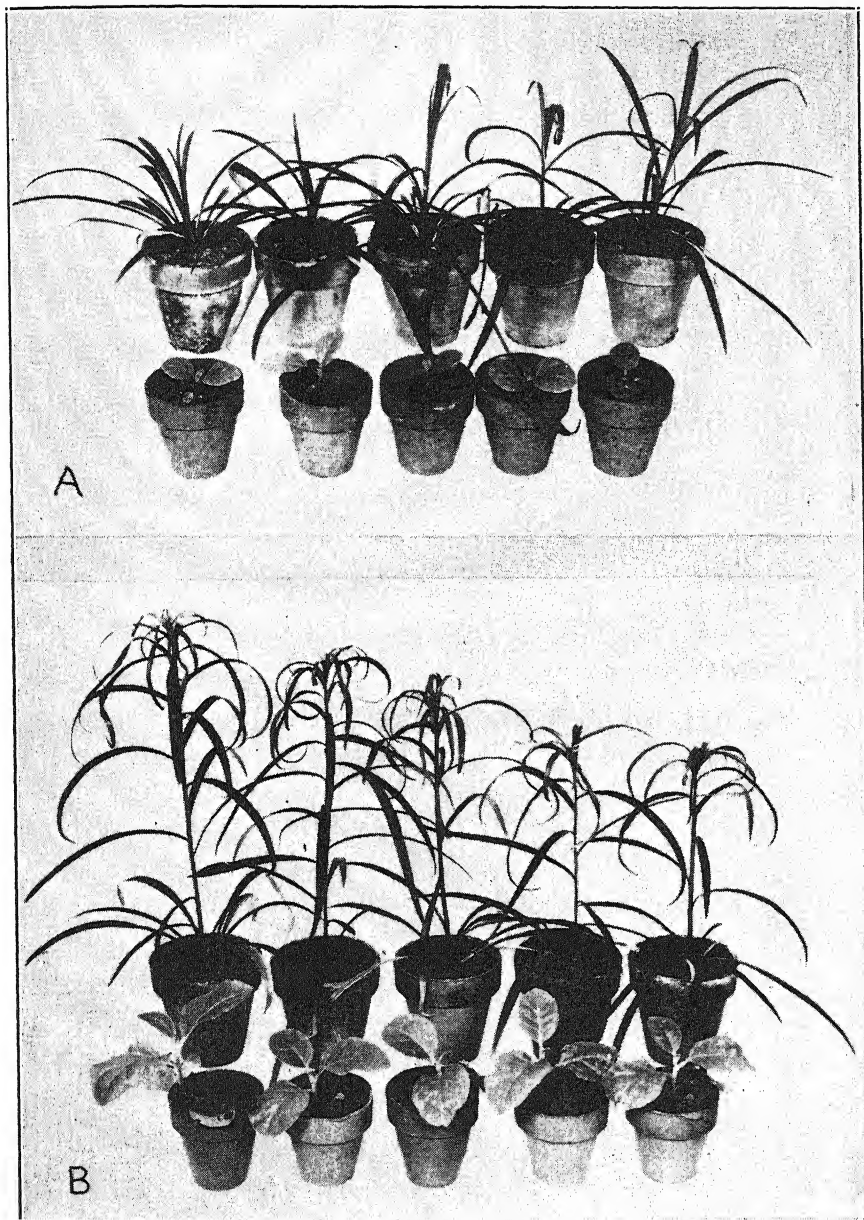


FIG. 2. Test plants used in detecting tulip and cucumber virus in lily. *Liliun formosanum* seedlings in 4-inch pots and Turkish tobacco in 3-inch pots. A. Representative set at stage suitable for test. B. A set 9 days after inoculation from a plant of the hybrid lily George C. Creelman, showing symptoms of tulip virus in *L. formosanum* and of cucumber virus in tobacco.

was detected the mean number of *Liliun formosanum* plants showing symptoms was $4.14 \pm .15$. In 27 trials in which cucumber mosaic was found, the

Species or variety	Source locality											
	Yonkers, N. Y.	White Plains, N. Y.	Charlotte, Vt.	Ottawa, Canada	Ithaca, N. Y.	Geneva, N. Y.	Beltsville, Md.	Thurmont, Md.	Aspen, Md.	Bellingham, Wash.	Bellevue, Wash.	Covallis, Oreg.
Anabile luteum	T	T			2C+T 1C	T H	H		H	H	T	
Auratum and var. ^a					C							
Backhouse hybrids					1C+T 1C	T						
Brownii			10H		10H							
Canadense (incl. var.)				H	2T 10H							
Candidum		C+T	C+T	T				T				
Cernuum						T						
Chalcedonicum					C+T							
Croceum					H							
Davidi (incl. Willmottiae)			C				H			H		
Davnotiae												
Dauricum luteum												
Elegans (and seedlings)												
Formosanum						2T						
George C. Creelman												
Giganteum	T	C+T		T	2H	10H						
Hansonii	T		12H	10H								
Hansonii-Marhan hybrids	T			5H								
Henryi	H		5H									
Leucanthum		H										
Maxwellii		C			C							
Monadelphum												2T

mean number of Turkish tobacco plants infected was $4.70 \pm .14$. Since the general level of infection is high in those sets that proved positive, some confidence may be placed in the significance of negative readings.

The results of these garden-lily trials are summarized in table 2. Tulip virus was detected in 31 species or varieties of lilies in 41 indexed, and from 13 localities in 15 sampled. Cucumber virus was found present in 18 species or varieties from 9 localities. Cucumber virus was not detected in 22 collections representing 6 localities in the West. Possibly the later season of indexing (July 28 to August 8), or the longer time in transit, was unfavorable for detecting cucumber virus in western samples, but the result is a striking one. Cucumber virus was found occurring alone in 11 index trials representing 10 species and varieties and 6 localities.

Twenty-two collections, including plants of 14 species or varieties from 7 localities, were found carrying both tulip and cucumber viruses. Some of these double infections were found in crook-neck *Lilium auratum* Lindl. and similarly affected *L. superbum* L.; in other instances *L. regale* Wils., *L. sargentiae* Wils., *L. tigrinum* Ker, and *L. umbellatum* Hort. carried virus of both classes with less damaging effects, although usually somewhat dwarfed.

Several of the bulb-propagated species, commonly assumed to be carriers of virus, were sampled in these trials. *Lilium candidum* L. in 6 samples carried tulip virus, in 2 samples both tulip and cucumber viruses, and 10 plants of recent seed origin were found virus-free. The hybrid George C. Creelman carried tulip virus in 4 localities and both viruses in 1 locality. *L. elegans* Thunb., of supposed hybrid origin, and commonly represented by named varieties, carried tulip virus in all 5 samples tested. The hybrids Maxwill, Princeps, T. A. Havemeyer, and *L. testaceum* Lindl., were all affected with one or more viruses in 8 tests. *L. tigrinum* was found affected with tulip virus 9 times, with both types once, and tested virus-free 8 times in samples from 7 localities. *L. tigrinum* was found apparently healthy in isolated gardens and in material recently collected from such isolated sites. *L. umbellatum*, of supposed hybrid origin and, like *L. elegans*, commonly represented by named varieties, carried one or both viruses in 5 samples, and was apparently healthy in 2 trials.

Lilium hansonii Leichtl., its hybrids Marhan (*L. martagon album* \times *L. hansonii*), and the Backhouse hybrids (Martagon-Hansonii hybrids), were uniformly virus-free in 9 tests that represented 50 individual plants from 5 localities. This species, as well as its hybrids, is vegetatively propagated, and is known as a type not subject to mosaic. These tests show that it is not commonly a symptomless carrier but may be resistant to infection or may tend to escape.

No virus was detected in leaves from bulbs of *Lilium myriophyllum superbum* (Baker) Wils. and *L. nepalense* D. Don, grown in a greenhouse at Geneva, New York, and said to be collected from the wild in India. This finding is in agreement with a general view that bulbs from the wild are virus-free.

A few conclusions may be drawn from the data available here. The existence of apparently virus-free *Lilium tigrinum* is in itself some assurance that isolation may be effective in protecting lilies from virus infection. Moreover, where isolation of garden-lily seedlings has been conscientiously attempted, it appears successful, as far as our sampling and indexing can be considered conclusive. Isolation has proved effective thus far in protecting our plantings of *L. longiflorum* seedlings at Charleston, South Carolina, for 2 years and at Los Angeles, California, for 1 year. The consistent failure to detect virus in *L. hansonii* and its hybrids, even when long grown in close proximity to diseased lilies, indicates that this species carries either resistance to or some tendency to escape from mosaic. The detection of double infections in *L. candidum*, George C. Creelman, *L. regale*, and *L. sargentiae* hybrids at White Plains, N. Y., in a private-garden collection, long carefully maintained at a high level of performance, suggests that there is not a close correlation between double infections and poor performance in all species. It may be inferred further that hybrids of the Regale-Sargentiae class may be carriers of complexes, as well as single virus infections, without showing symptoms that would commonly call for roguing.

These index tests show that both tulip virus and cucumber virus are involved in mosaic of garden lilies. As in Easter lilies, the tulip virus is more commonly found. The occurrence of 2 distinct viruses in garden lilies greatly complicates problems involving resistance, tolerance, or capacity to escape infection. Obviously, the symptoms found in lilies in nature must be reproduced in inoculation experiments before the viruses isolated can be established as the sole causal agents. Determination of the reactions of the various species and varieties to each and to both viruses will be a long and tedious undertaking. In the meantime the culture of lilies from seed under suitable isolation offers promise as a solution of the problem of virus-disease control. Those who attempt to grow virus-free lilies by this method must face the fact that either tulip virus or cucumber virus may occur in latent form in appropriate species that have not been suitably isolated.

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THE RELATION OF TEMPERATURE TO COMMON AND HALO BLIGHT OF BEANS¹

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Inoculation experiments with common blight of beans (*Phaseolus vulgaris* L.) caused by *Phytophthora phaseoli* (E.F.S.) Bergey *et al.* and halo blight caused by *Phyt. medicaginis* var. *phaseolicola* Burkh. have resulted in considerable variation in the percentage of infection, time of appearance of symptoms and the type of symptom occurring. This variability has been evident both in the field and in the greenhouse, where conditions were more uniform. Differences in the age and variety of bean plants being tested failed to adequately explain the variability. It was also observed that these diseases sometimes occurred in commercial fields with apparent suddenness and under conditions that did not seem especially favorable.

The literature contains many statements based on observational evidence concerning the conditions most favorable for the two diseases. In general, common blight is considered as a high-temperature disease. Burkholder (1) observed the disease was more prevalent in hot than in cool seasons and that infection was more easily obtained in the greenhouse at 80° than at 65° F. He noted that at the latter temperature the leaf spots developed very slowly. From observational evidence he concluded that, "The pathogene progresses more rapidly in the plant tissue at high temperature than at low." Halo blight is generally considered a low-temperature disease. Burkholder (1) describes two types of leaf spots, the halo spots, which he found to occur during cool weather, and small, numerous angular lesions without a definite halo, which he observed to occur later in the season, during hot weather. He considered the lack of a halo to be due partly to the fact that the whole leaf became chlorotic.

Moisture usually is considered as being essential for both diseases, although Muncie (3) states that common blight may spread rapidly during dry weather. Higgins (2) considers moisture to be essential for infection with halo blight, and points out that "Infection is very materially reduced and often entirely inhibited if the plants are allowed to dry to the wilting point even three or four days after inoculation." Zaumeyer (4) tested the effect of high moisture, both before and after inoculation, by the use of moist chambers. He found that very little infection occurred with either disease unless the moist chambers were used for 24 hours after inoculation.

Because of the lack of published, experimental evidence the following experiments were undertaken to establish more accurately the effect of temperature upon infection and upon the subsequent development of these diseases. A few tests were included to determine the possible effect of rela-

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tive humidity upon the development of these diseases after infection had occurred.

METHODS

The experiments were carried out in electrically heated, double-wall glass cases in a refrigerated greenhouse. These cases were placed over water baths and an electric fan circulated the air over the water, so that a high relative humidity could be maintained. In some of the experiments a contrasting low relative humidity was obtained by eliminating the water bath.

Because of their known susceptibility to both bacterial blights, Red Kidney beans, obtained from a blight-free area, were used in all of the tests. These were grown in galvanized boxes or cans and the soil moisture was kept near the optimum as judged by the appearance of the plants. In each test the plants were selected for uniformity of size and development, the first trifoliate leaf being well developed and the second and third just unfolding.

The plants were placed in the high-humidity cases for 24 hours before being inoculated. The upper and lower surfaces of the leaves were then thoroughly sprayed with a bacterial suspension made up of a mixture of strains of the organism to be tested.

Noninoculated controls were not included in the tests conducted in the temperature cases, as it was considered that the leaves produced after the plants had been inoculated would serve as an adequate check on the effect of temperature on the foliage. These leaves almost invariably remained free from blight. Additional plants from the same seed source were always grown in another greenhouse at the same time and always remained free from both halo and common blight. The bean plants grew slowly at 12° C. but the foliage appeared normal. The best growth occurred at temperatures of 16°, 20°, and 24° C. There was considerable etiolation at 28° and very poor growth with considerable yellowing at 32° C.

RESULTS

Relative Humidity. In the first experiments with each disease, after an incubation period of 24 hours at a high relative humidity, one-half of the plants at each temperature were removed to a low-relative-humidity case held at the same temperature.

Low humidity with common blight caused the infected area of the leaf to dry out more rapidly and the area affected was greater than at high humidities. This greater severity of the symptoms with low humidities occurred at all of the temperatures tested. In contrast with common blight, the low humidities had no apparent effect on the development of halo-blight symptoms. Even at the high temperatures, where the foliage sometimes reached the wilting point in the low humidity cases, the severity of infection was as great as with high humidity. This is in contrast to the results reported by Higgins (2).

Temperature. Common Blight. In the experiments referred to above it was found that temperature had a very marked effect on the length of the

incubation period with common blight. The first symptoms appeared in 6 days at 32°, 10 days at 28°, 14 days at 24°, and no symptoms were evident at the end of 17 days at 20° and 16° C. It was at first thought that no infection had occurred at these low temperatures, but, when, after 17 days, the plants at 20° were transferred to 32° C., large blighted areas appeared on the leaves in 2 days. Those at 16° were transferred to 28° C. and symptoms appeared 7 days later. The rapidity with which the symptoms developed when the plants at 20° were transferred to 32° C. clearly indicated that infection had occurred at the lower temperature, but that the symptoms were masked. In addition to the longer incubation period, the symptoms were always less severe at the low temperatures, particularly on the primary leaves. It was noticeable at all temperatures that infection was more prevalent on the older leaves than on those that were just unfolding at the time of inoculation.

The results of this first experiment suggested that the symptoms would have appeared if the plants had been held longer at 16° and 20° C. Accordingly, the experiment was repeated several times for longer periods, using only the high relative humidity chambers. The results presented in table 1 are of a typical experiment. Symptoms developed at 20° after 23 days

TABLE 1.—*Relation of temperature to common blight*

Temperature	Number of plants	Days to first symptoms	Infection of primary leaves				Infection of trifoliolate leaves ^c			
			None	Slight	Medium	Severe	None	Slight	Medium	Severe
° C.	Number	Number	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
32	14	7	35	38	23	4	65	17	12	6
28	23	9	46	31	13	10	59	21	13	7
24	24	16	73	23	4	0	47	29	13	11
20	12	23	96	4	0	0	55	34	8	3
20-32 ^a	11	23 ^a	68	32	0	0	40	20	10	30
16	12	27	100	0	0	0	94	6	0	0
16-32 ^b	9	27 ^b	92	6	0	0	74	21	5	0

^a The temperature was changed from 20° to 32° C. 23 days after inoculation at which time there were no symptoms on the primary leaves and only 12 per cent of the secondary leaves showed slight infection.

^b The temperature was changed from 16° to 32° C. 23 days after inoculation at which time there were no symptoms. The symptoms appeared 4 days after changing to 32° C.

^c Records are based on final readings made 27 days after inoculation on the first 3 trifoliolate leaves. The degree of severity indicates the relative amount of infected tissue per leaf.

and a few symptoms appeared on plants held at 16° C. after 27 days. The symptoms of plants transferred from these temperatures to 32° rapidly increased in severity, although those originally held at 16° C. never developed symptoms so severe as those at higher temperatures during the duration of the experiment (27 days).

The causal organism was consistently isolated from leaves showing symp-

toms including those where the symptoms had previously been masked by low temperatures.

The above experiments were all conducted with the Red Kidney variety. It is possible that other varieties might vary in their response to temperature, but a few tests made with the Great Northern variety of field beans yielded results similar to those reported for the Red Kidney variety.

Halo Blight. In the preliminary experiments the first symptoms of halo blight appeared in 6 to 10 days after inoculation at 24° and 28° C. Usually the symptoms appeared 2 to 3 days later at the lower temperatures, but the effect of temperature on the incubation period was very slight as compared with common blight. As with common blight, there was considerable variation in the percentage of leaves infected at the different temperatures, but no consistent differences could be noted within a single experiment.

The effect of high temperature was chiefly confined to a modification of the symptoms and to an increase in the number of infection points per leaf. As previously stated, the relative humidity after a 24-hour period following inoculation had no effect on the development of the disease. The experiment was repeated several times, using a high humidity at all temperatures, and the effect of temperature was similar in all experiments. The results of 3 such tests are combined in the data presented in table 2. The amount of

TABLE 2.—*Relation of temperature to halo blight*

Temperature	Number of plants	Infection of trifoliolate leaves ^b				Occurrence of halo symptom ^c
		None	Slight	Medium	Severe	
° C.	Number	Per cent	Per cent	Per cent	Per cent	
32	46	55	10	11	24	none
28	48	60	18	13	9	none
24	63	66	19	11	4	small
20	63	64	28	5	3	large
16	62	58	32	7	3	large
12 ^a	21	77	18	3	2	large

^a Plants grown at 12° C. were from a different experiment but were grown in a comparable manner.

^b The data are based on the trifoliolate leaves exposed at the time of inoculation and the results of three experiments are averaged. The degree of severity indicates the relative number of infections per leaf.

^c See figure 1 for type of infection.

infection on the primary or cotyledonary leaves was very small as contrasted with that occurring with common blight. Because of this small amount of infection and the lack of consistent differences, the data on primary leaves are omitted from the table.

The two outstanding effects of temperature were the increased number of infections per leaf at high temperatures and the variation in the type of symptoms. In table 2 it can be seen that a higher percentage of leaves was listed as severely infected at the high temperatures. The degree of severity was based on the number of infections.

The typical halo surrounding the infected area occurred only at the lower

emperatures, 12°, 16°, and 20° C., at all of which temperatures there were typically few infection points per leaf, but the light green-yellow halo often involved one-fourth or more of the leaf area. At 24° the halo was quite mall, and at 28° and 32° C. there was no halo visible to the naked eye. The ymptoms at these two high temperatures on Red Kidney plants were so typical that they could easily be mistaken for insect injury. The spots vere usually less than a millimeter in diameter on the upper surface and

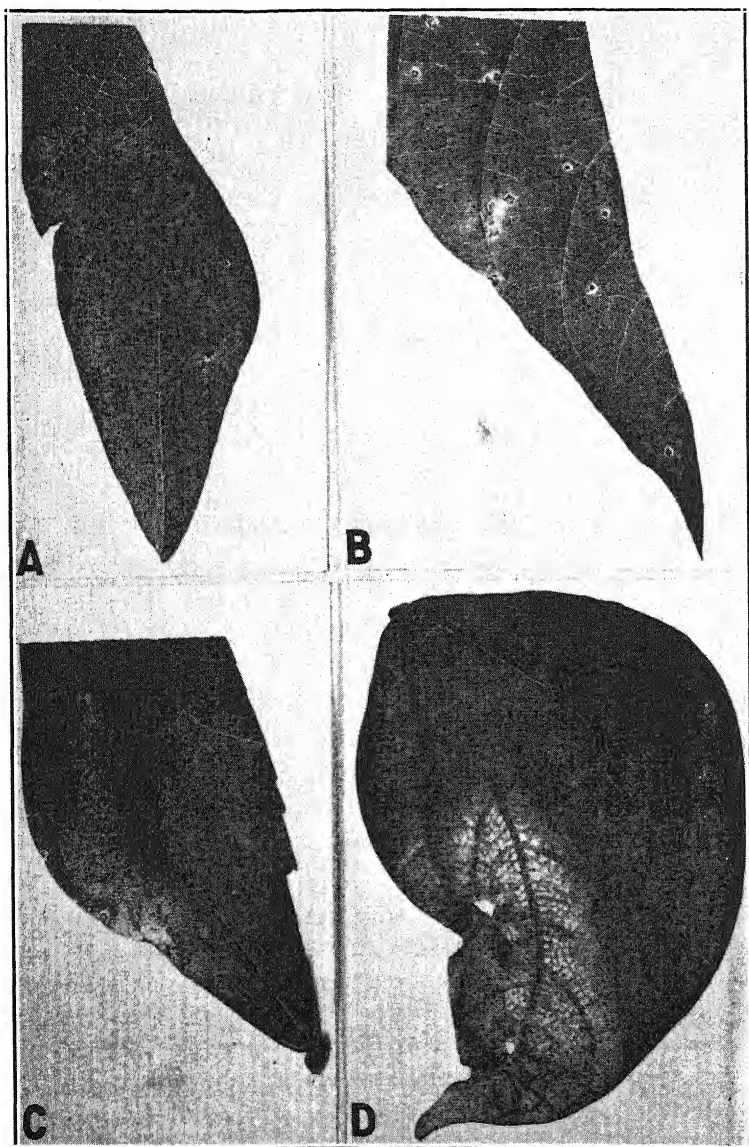


FIG. 1. The effect of temperature on the symptoms of halo blight of beans. A, B, and C. Portions of inoculated leaves at 28°, 24°, and 20° C., respectively. D. Inoculated

slightly larger on the under side of the leaf. When magnified a very narrow band of chlorotic tissue could be seen (Fig. 1) on the upper side of the leaf, but it in no way resembled the halo that occurred at the lower temperatures. Bacterial exudate was usually present on the under side of the infected spots. Figure 1 shows typical examples of the variation in symptoms occurring at different temperatures. The causal organism was repeatedly isolated from the bacterial exudate of these atypical spots, which occurred at high temperatures.

The lack of a halo around the infected tissue was not due to a general chlorosis of the leaf, as noted by Burkholder (1). Chlorosis often is associated with a very large number of infections per leaf, but no halo occurred at high temperatures when only one or two infections were present on a green leaf.

In all of the halo blight inoculations it was noted that the greatest number of infections occurred on the youngest leaves that were just unfolding at the time of inoculation. This was in contrast with common blight, where the most infection occurred on the older leaves.

One test was conducted in which U. S. No. 5 Refugee was compared with the Red Kidney. The effect of temperature on the symptoms was similar, but it is possible that other varieties might differ in their response to temperature.

DISCUSSION

While common blight can be termed a high-temperature disease, due to the rapidity with which a large amount of leaf tissue can be involved, it is clearly evident that infection can occur at relatively low temperatures for the bean plant and that the incubation period is greatly prolonged at these low temperatures. This delay in the appearance of symptoms might possibly explain some outbreaks of the disease under conditions usually not considered ideal for infection. The rapidity with which the symptoms develop when transferred from low to high temperatures indicates that this is a masking effect and not entirely a slowing up of the progress of the pathogen through the plant tissues, as stated by Burkholder (1). Further experiments are necessary to determine how much of the delay in the appearance of symptoms is attributable to a slower invasion and how much to the masking of symptoms in invaded tissue.

Halo blight, likewise, can occur over a wide range of temperatures. The occurrence of the conspicuous halo at the lower temperatures readily explains why this disease is considered to be favored by low temperatures. The actual number of infections, however, may be greater at higher temperatures, and each of these inconspicuous spots can serve as a source of inoculum for the further spread of the disease. It would be difficult to detect many of these lesions in a casual examination of plants in the field. An epidemic could be gradually built up in this way, so that, with later weather conditions favorable for the development of the halo symptom, one might believe a severe infection had occurred simultaneously in the entire field.

SUMMARY

Common blight of beans was produced by inoculation with *Phytomonas phaseoli* at all temperatures tested from 16° to 32° C. At low temperatures the incubation period was greatly prolonged. At 32° the symptoms appeared in 7 days, at 20° symptoms appeared in 23 days, while at 16° C. only slight symptoms appeared on a few leaves at the end of 27 days.

With common blight the transfer of plants from low to high temperatures caused the rapid development of symptoms on leaves previously appearing healthy. The amount of affected leaf tissue was greatest on the plants held constantly at the high temperatures. The oldest leaves of the young plants tested were more susceptible to infection than the young leaves just unfolding at the time of inoculation.

Halo blight was produced by inoculations with *Phytomonas medicaginis* v. *phaseolicola* at all temperatures tested between 12° and 32° C.

The conspicuous halo occurred chiefly at 20° and below and, occasionally, a slight halo was present at 24° C. At 28° and 32° C. no halo symptoms appeared, the infections were greater in number, but the spots were small and inconspicuous, although bacterial exudate was present on the under side of the leaves.

The youngest leaves, just unfolding at the time of inoculation, were the most susceptible to halo blight.

Very little infection of primary leaves occurred with halo blight, while, with common blight, they were seriously infected.

The relative humidity after a 24-hour incubation period at high humidity had no effect on halo blight, but low humidities increased the severity of the symptoms on leaves infected with common blight.

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SPORANGIAL PROLIFERATION IN PERONOSPORA TABACINA

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To date, no one has been able to grow members of the family Peronosporaceae, or downy mildews, on artificial media. In order to have available an adequate supply of sporangia for our studies of *Peronospora tabacina* Adam, frequent transfer of the pathogen from diseased to healthy seedlings proved necessary. These seedlings were grown in half-gallon glass fruit

jars containing approximately 500 g. of previously steam-sterilized soil. Seedlings were inoculated by atomizing a water suspension of sporangia upon them, and the jars were then closed with their screw-cap tops. The jars were then placed in a special incubation chamber maintained at a temperature of approximately 15° C. and continuously illuminated with a 25-watt bulb. Under these abnormal conditions, the pathogen produced proliferated sporangia. This phenomenon is recorded, for, so far as the writers are aware, it has not hitherto been recorded for any of the Peronosporaceae.

It should be borne in mind that *Peronospora tabacina* and other species of the genus possess dendritic, dichotomously-branched sporangiophores and that the sporangia are borne at the apices of curved, tapering branchlets. All sporangia of *P. tabacina* mature and are shed almost simultaneously, presumably being dislodged by air currents or by rain. Under usual conditions a crop of sporangia is produced at daybreak each morning, a response probably governed by light conditions. The sporangia range in size from $10.5\text{--}24 \times 10.5\text{--}22 \mu$, the average dimensions being $18.4 \times 15 \mu$ (13).

Under the conditions of sustained high relative humidity within the jars and of constant weak illumination to which the pathogen and seedlings were exposed, the diurnal rhythm in production of sporangia was interrupted. Instead of finding fully developed sporangia in the morning, it was noted that the sporangiophores emerging from the several adjacent stomates were in different stages of development, the terminal branchlets often being more than twice as long as normal. The sporangia varied greatly in size, remained attached to the sporangiophores, and several types of proliferation were in evidence. In some the inner sporangial wall protruded apically and became enlarged (Fig. 1, *a* and *b*). In others there was formed a tube that either remained unbranched or branched dichotomously, developing small secondary terminal sporangia (Fig. 1, *c*, *d*, and *g*). In consequence a primary sporangium might give rise to 1 to 4 secondary sporangia, each smaller than the primary sporangium. As indicated in figure 1, *f*, the secondary sporangia also may proliferate, and tertiary sporangia would, no doubt, eventually be formed in such cases. Proliferation of another type is shown in figures 1, *h*, wherein the primary sporangium has produced a sporangiophore, dichotomously-branched and bearing eight diminutive, immature, secondary sporangia.

Proliferation apparently is causally related to the continuously high relative humidity and subdued illumination to which the pathogen was subjected. This opinion is supported by the fact that during the 8 years in which this fungus has been studied proliferation has never been noted on naturally infected plants grown out-of-doors.

In other fungi closely related to *Peronospora tabacina*, including members of the families Saprolegniaceae and Pythiaceae, the phenomenon of proliferation is not of unusual occurrence. In the former family, among species of *Saprolegnia*, new sporangia may form repeatedly within the basal

portion of the old sporangium; in the latter family a similar condition exists in the case of *Pythiomorpha gonapodioides* Petersen (8). This type of proliferation involves the portion of the hypha immediately beneath the sporangium and not the sporangium itself. Kanouse (6) observed that in *P. gonapodioides* the apex of the sporangiophore may be rejuvenated repeatedly, resulting in series of sporangia arranged nest-like or in a row. Butler's

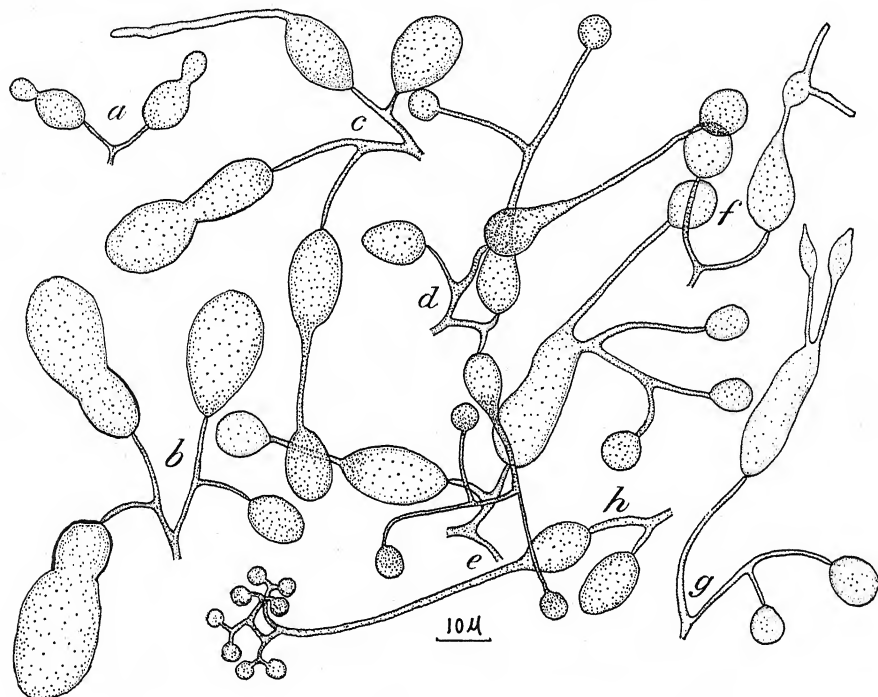


FIG. 1. *a*. Two dwarfed sporangia, each of which has budded in a yeast-like manner. *b*. Four sporangia, one dwarfed, one of normal size, and two approximately twice the normal length, whose inner wall has become extended from the sporangial apex. The sporangiophore tips are abnormally elongated. *c*. Group of four sporangia, one of which has sprouted preparatory to forming a secondary sporangium, and another of which is forming a secondary sporangium. *d*. One sporangium has grown to form a secondary sporangium, another is forming 2 diminutive sporangia, and another one is forming three tiny sporangia. *e*. Proliferation of sporangia one of which has produced a single sporangium, the other of which, abnormal in size and shape, has proliferated to form four sporangia. *f*. The secondary sporangium is itself proliferating. *g*. The secondary sporangia borne by one elongated sporangium in process of formation. *h*. One sporangium that has proliferated to form a sporangiophore on which eight sporangia are forming.

plate 3, figure 4 (1) shows, in *Pythium proliferum* de Bary, a series of proliferating sporangia growing through and one above the other.

Another type of proliferation, involving sporangiospores instead of sporangia, is exhibited by the repeated emergence of laterally-ciliate zoospores in *Dictyuchus* sp., described by Weston (12), in *Achyla racemosa* Hildebrand, described by Höhnk (4), in *Phytophthora infestans* (Mont.) de Bary, described by Murphy (7), in *Pythium proliferum* de Bary, described

by Cornu (2), and in *Pythium diacarpum* Butler described by Butler (1). Still another type of repetitional development, in which the sporangiospore germinates, forming at the tip of the germ tube a new sporangium, which emits only one zoospore, occurs in *Pythium dictyosporum* Racib. (9) and in *Phytophthora* sp., associated with pink rot of potato (3).

Primary sporangia may produce tubes surmounted by secondary sporangia in certain species of *Phytophthora*. Two secondary sporangia are thus formed by *Peronospora cactorum* (Cohn et Leb.) Schroet. shown in Rosenbaum's¹ figure 16 (10). Another of his illustrations in the same figure shows that tertiary sporangia may arise. Proliferation of this type with production of a single secondary sporangium was noted in *P. infestans* by Jones, Giddings, and Lutman (5: Fig. 6) and in *P. phaseoli* Thaxter by Thaxter (11: Pl. 3, figs. 31-32). These species may, therefore, exhibit a proliferative development analogous with that herein noted in *Peronospora tabacina*.

Apparently, proliferation of the kind illustrated in figure 1, *h*, finds no counterpart among the related families of Phycomycetes. In the Peronosporaceae all of the protoplasmic content most commonly migrates from the sporangiophore into the sporangia when the sporangia form, leaving the sporangiophore empty. Presumably, differentiation does not occur during migration of the protoplasm into the sporangia, so that each sporangium is totipotent. Repetitional development of this type could therefore be anticipated to occur. Sufficient explanation of the function of the other types of proliferation would appear to require only the statement that the fundamental law of life of each organism is reproduction of its kind.

Höhnk (4) pointed out that there is a tendency from planetism to aplanetism among the Saprolegniaceae, correlated with progression from water forms toward land forms. Conceivably, polyplanetism, well known among the Saprolegniaceae, and proliferation may be parallel developments, and both may have phylogenetic significance.

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¹ Fitzpatrick's figure 72g, and also d, e, f, n, and u, in *The Lower Fungi—Phycomycetes*, are erroneously stated to be taken from Rosenbaum's "Studies of the genus *Phytophthora*." Jour. Agr. Res. [U.S.] 8: 233-276, 1917.

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RELATION OF STOMATA TO INFECTION OF TOBACCO LEAVES BY BACTERIUM TABACUM¹

STEPHEN DIACHUN

(Accepted for publication October 23, 1939)

One of the common portals of entry for bacteria that cause leaf spots is the stoma (6, 9). It is believed that in natural field infections *Bacterium tabacum* Wolf and Foster usually invades tobacco leaves through stomata (1, 3, 5). When leaves are artificially inoculated by atomizing with a bacterial suspension the bacteria presumably enter through stomata. Clinton and McCormick (3) reported that when immature tobacco leaves were sprayed, infections rarely occurred, indicating that "entrance takes place through the stomates, which in these leaves are not so fully developed or liable to be open."

On several occasions, both in the field and in the greenhouse, when leaves were atomized with bacterial suspension late in the afternoon or on dull, cloudy days, only a very limited amount of infection resulted. Diachun and Valteau (4) have reported that stomata of leaves of greenhouse tobacco plants are usually wider open during the day than at night, and on sunny days than on very dull days. Subsequent field tests have shown that, in general, the results obtained on greenhouse plants are applicable also to field-grown plants.

It is the purpose of this paper to report results of experiments designed to determine the effect of stomatal opening at the time of inoculation on infection of tobacco leaves inoculated by atomizing with *Bacterium tabacum*.

EXPERIMENTAL RESULTS

To determine whether the degree of stomatal opening, and, consequently, the time of inoculation, influences the amount of infection, the following tests were made. On January 7, 1939, 6 leaves of each of 3 vigorous white Burley plants were atomized by means of a de Vilbis atomizer with a 24-hour broth culture of *Bacterium tabacum* diluted with 10 parts of sterile water.

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

The nozzle of the atomizer was held 2 inches from the lower surface. Plant A was inoculated in the greenhouse at noon. Plant B was placed in a dark room at 9 a.m., inoculated in the dark room at noon, and kept there until 6 p.m. Plant C, which was in the greenhouse all day, was inoculated at 6:30 p.m., more than an hour after twilight. At the time of inoculation several pieces of epidermis were stripped from each plant for stomatal examination, using Lloyd's technique (8). To compare the stomatal opening of leaves on the 3 plants an index expressed as K^2 was used. The value of K for plant A was .32; for plant B, .13; and for plant C, .14. That is, the stomata on plant A were more than twice as wide as those on plants B and C. By January 16, plant A was heavily infected. There were only 15 isolated wildfire spots on plant B, and on plant C only 4 such spots. This experiment was repeated 5 times with similar results: the stomata were open on plants inoculated in the greenhouse at noon and infection was severe; they were nearly closed on plants inoculated at night and in artificial darkness, and infection was limited (Fig. 1).



FIG. 1. Effect of stomatal opening on infection with *Bact. tabacum*. The leaves were atomized with a 24-hour broth culture diluted with 10 parts of sterile water. The nozzle of the atomizer was held 2 inches from the lower surface. Inoculated February 13, 1939, and photographed February 20. A. Inoculated in the greenhouse at 2:30 p.m. on a sunny day, stomata open, K .25. B. Placed in dark room at 11:30 a.m., inoculated in dark room at 2:30 p.m., stomata nearly closed, K .05. C. Inoculated in the greenhouse at 7 p.m., stomata nearly closed, K .06.

Inoculations were made also in the field on several occasions when stomata were closed. Infection was always less severe when stomata were closed than when they were open. For example, on August 7, 1939, the left side of 2 leaves was inoculated at 9 p.m., when the stomata were nearly closed, K being .05. The right side of the same leaves was inoculated at 6:15 the next morning when the stomata were open, K being .25. Both sides were inoculated by atomizing with the atomizer 2 inches from the lower surface. By August 15 there was good infection on the right side and only a very few spots on the left side of each leaf. Again, on August 8, the

² K is the ratio of the average actual width to the average potential length of the opening of 20 measured stomata.

right side of a leaf of a dark tobacco plant was inoculated by atomizing at 4 p.m. The lower surface of the leaf was exposed to direct sunlight and the stomata were nearly closed.³ The left side of the same leaf was inoculated at 9 the next morning, when the stomata were open. By August 15 there was good wildfire infection on the left side and but little on the right side of the leaf (Fig. 2).

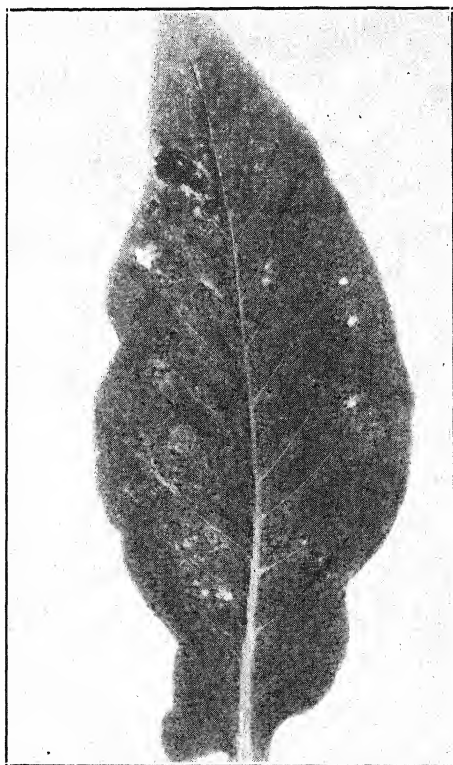


FIG. 2. Effect of stomatal opening on infection of a field-grown plant with *Bacterium tabacum*. Both sides of the leaf were atomized with the nozzle of the atomizer 2 inches from the lower surface. The right side was atomized at 4 p.m., August 8, when stomata were nearly closed. The left side was atomized at 9 a.m., August 9, when stomata were open. Photographed, August 21, 1939.

DISCUSSION

The results of the experiments reported here call attention to the fact that when leaves of tobacco, and perhaps other plants, are to be inoculated with bacteria by atomizing, the condition of the stomata must be considered. Erroneous conclusions about the resistance or susceptibility of plants being compared, or about the virulence of cultures under test may be reached unless care is taken to make all inoculations when stomata are known to be open. Leaves that are shaded, wilted, or turned up with the lower surface

³ It was observed frequently that when leaves were turned up, so that the lower surface was exposed to the afternoon sun, the stomata were closed.

exposed to the sun, should be avoided, for observations have shown that stomata are likely to be closed under these circumstances.

It is generally believed that there is a close correlation between outbreaks of wildfire and stormy, rainy weather. Chapman and Anderson (1) have said, "It has been noted by all investigators of the disease and by tobacco growers that rapid spread invariably follows heavy rains. . . . These two agents (wind and rain) are undoubtedly the most potent of all factors involved in dissemination." Some workers have believed that heavy storms produce wounding, which facilitates invasion. J. Johnson and Fracker (7) reported that storms, especially beating rains, "have a very important relation to wildfire in that they favor infection to a high degree. The bacteria are often unable to infect leaves except through slightly wounded tissue such as may be produced by beating rain." More recently, Clayton (2) has expressed the belief that storms are important in connection with water-soaking. He feels that the occurrence of epidemic wildfire is "conditional on storms of sufficient severity and duration to produce and maintain water-soaked areas on the leaves." Unpublished observations of W. D. Valleau and E. M. Johnson indicate that, although outbreaks of wildfire are often associated with rainy, stormy weather, every storm does not contribute to infection and spread. From the experiments reported here it is concluded that atomizing tobacco leaves with *Bacterium tabacum* produces infection only if the stomata are open. The suggestion occurs that perhaps natural infection by bacteria carried in windblown rain may occur only when stomata are open. It has been observed that during some rainstorms stomata are open; during others, they are closed, depending perhaps on the light intensity. On July 6, 1939, at 3:30 p.m. there was a light shower; light was 800 foot-candles; stomata were open, K being .24. On July 20 it rained intermittently all morning; light was 600 foot-candles; stomata were open, K being .23. On July 28 it rained at 4 p.m.; stomata were open, K being .16. On July 29 it rained from 8 to 10 a.m.; light was 300 foot-candles; stomata were open, K being .25. However, on July 19, 1939, when it rained at 12:30 p.m., the light was only 100 foot-candles, and stomata were closed, K being .05. It cleared up in a short time; at 2 p.m. light was 2000 foot-candles, and stomata were open on some of the leaves. At 4:30 p.m. it rained again, light was less than 100 foot-candles, and stomata were closed. Although there was little wind with these rains, it is conceivable that high winds accompanying such rains could dash drops of water with bacteria against leaves with sufficient force to drive some of the water into the leaf if the stomata were open, while similar storms might not produce infection if the stomata were closed.

SUMMARY

The experiments reported here indicate that in the greenhouse or field the stomatal condition of leaves is a factor determining the amount of infection on leaves atomized with a suspension of *Bacterium tabacum*. During

the day the stomata are usually open, and atomizing produces heavy infection on tender leaves of vigorous plants. At night or in artificial darkness stomata are closed or nearly so, and leaves atomized with *Bacterium tabacum* develop only a limited amount of infection (a few open stomata were always observed, even on plants in the dark).

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ATTEMPTS TO ISOLATE CERATOSTOMELLA ULMI FROM STORED ELM WOOD

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(Accepted for publication Nov. 22, 1939)

Ceratostomella ulmi (Schwarz) Buisman, the fungus causing the Dutch elm disease, is known to survive the death of the host long enough to contaminate elm bark beetles emerging from infected dead wood. The present study was carried on in the hope of learning more about the longevity of *C. ulmi* in cut elm wood and some of the field conditions affecting it.

In late September, 1936, infected living branches 1 to 4 inches in diameter were cut from several American elms affected by the Dutch elm disease. The branches were cut into 611 one-foot lengths and divided into 3 approximately equal lots for storage under different field conditions. Each lot was so selected as to include about one-third of the sticks from each tree. Lot 1 was placed on the ground in deep forest shade. Lot 2 was laid on the grass in an area unshaded, except for weeds which grew up and were cut during the middle of the summer. Lot 3 was placed on a rack 18 inches above the ground, where the sticks were in direct sunshine for the greater part of the day. All bark was removed from half of the pieces in each lot and peeled sticks were placed in groups beside the non-peeled in each of the three environments. The side of each diseased stick, showing at the cut ends the most severe discoloration indicative of the Dutch elm disease, was marked

for subsequent culturing. The pieces in each lot were laid so that the marked side of every other one was on top, while that of the alternate sticks was placed downward. Unfortunately, there was not time to attempt to isolate the fungus from each stick at the outset of the experiment. The distribution of the diseased wood is summarized in table 1.

TABLE 1.—*Recovery of Ceratostomella ulmi from diseased elm wood stored variously*

Environment of stored sticks, position of discoloration cultured, and condition of sticks	Number of diseased sticks	Percentage of sticks cultured yielding <i>C. ulmi</i> after—	
		4 months	20 months
WOODS:			
Discoloration down—			
Bark off	68	73	2
Bark on	69	95	23
Discoloration up—			
Bark off	63	29	0
Bark on	62	70	8
GRASS:			
Discoloration down—			
Bark off	67	39	0
Bark on	63	62	2
Discoloration up—			
Bark off	62	17	0
Bark on	51	43	0
RACK:			
Discoloration down—			
Bark off	59	35	4
Bark on	66	86	2
Discoloration up—			
Bark off	64	33	0
Bark on	67	87	0

Fifty similar pieces of non-diseased wood, 25 peeled and 25 non-peeled, were stored in each of the 3 environments as checks.

In January, 1937, 4 months after the experiment was begun, an attempt was made to isolate *Ceratostomella ulmi* from one-third of the total number of sticks so chosen as to represent each condition of storage. After attempted isolation, the sticks were returned to their previous environments. In May, 1938, 20 months after the initiation of the experiment, an attempt was made to isolate the fungus from all the sticks. The experiment was then concluded. On both dates isolation was attempted in two ways by placing chips cut from the wood and flooded briefly with hydrogen peroxide (a) into Petri dishes containing potato-sucrose agar, and (b) into Petri-dish moist chambers, where they were incubated for 3 weeks at approximately 60° F. If, during incubation, coremia developed on the chips, some of their spore-containing exudate was transferred to potato-sucrose agar to determine with certainty whether the coremia were those of *C. ulmi* or of closely related species that form somewhat similar coremia.

Table 1 shows the percentages of the total numbers of cultured diseased sticks stored in each manner from which *Ceratostomella ulmi* was recovered after 4 and after 20 months. None of the check sticks yielded *C. ulmi* when cultured. While the mathematical significance of the data in table 1 has not been tested, inspection of them leads to the following conclusions: (1) With the passage of time, *C. ulmi* was recoverable from fewer sticks. After four months' storage only a part of the sticks cultured which were stored under each condition yielded the fungus. After 20 months, *C. ulmi* was recoverable from only a small percentage of all sticks, except those stored in the woods with the bark on and the side marked for culturing oriented downward. Twenty-three per cent of these still yielded *C. ulmi* when cultured after 20 months' storage. (2) Peeled sticks yielded *C. ulmi* in consistently lower percentages than non-peeled sticks after 4 months under all storage conditions. After 20 months the differences between peeled and non-peeled sticks were slight or negligible, except for those sticks stored in the woods with the side marked for culturing oriented downward. Under these conditions only 2 per cent of the peeled sticks yielded *C. ulmi*, while the fungus was recovered from 23 per cent of the non-peeled sticks. (3) The effects of the place of storage and orientation of the side marked for culturing were shown with less consistency and were in many cases small or negligible.

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PHYTOPATHOLOGICAL NOTES

Selenized Soil as a Control for Aphids and Red Spiders on Sorghum in the Greenhouse.—Greenhouse studies on sorghum diseases are frequently complicated by infestations of aphids, *Aphis maidis* (Fitch), and red spiders, *Tetranychus telarius* (L.), to both of which pests sorghum is very susceptible. The effects of aphid and red-spider infestation on the plants frequently mask the symptoms of the disease being studied. Frequent fumigations and sprayings necessary for the control of these pests are laborious, expensive, and sometimes injurious to the plants.

Hurd-Karrer and Poos¹ in 1936 controlled aphids on wheat, oats, rye, and barley in the greenhouse by adding 10 p.p.m. selenium as sodium selenate to the soil; both aphids and red spiders were controlled on wheat plants grown in nutrient solutions containing from 1 to 3 p.p.m. of selenium. Morris *et al.*² reported control of red spiders on corn grown in nutrient solutions to which 1 p.p.m. selenium was added weekly.

In September, 1939, the writer grew sorghum in 4 sections of a green-

¹ Hurd-Karrer, A. M., and F. W. Poos. Toxicity of selenium-containing plants to aphids. *Science* 84: 252. 1936.

² Morris, V. H., C. R. Neiswander, and J. D. Sayre. Toxicity of selenium-containing plants to red spiders as a means of control. (Paper presented before the 16th annual meeting of the American Society of Plant Physiologists December 28-30, 1939, at Columbus, Ohio.)

house bench filled with Keyport clay loam to which had been added 0, 5, 10, and 15 p.p.m., respectively, of selenium in the form of sodium selenate, which was thoroughly mixed into the whole mass of soil. Emergence was not affected by the selenium, but, after 2 weeks, the average height of the plants was reduced by the 3 selenium concentrations 19, 43, and 50 per cent, respectively, compared with that of the control without selenium. All attempts to infest these plants with aphids or red spiders were futile. The controls, however, became severely infested and were badly damaged. In a second planting in these same sections 3 months later, a reduction of 5 to 25 per cent was observed in the height of the plants in the selenized soil, and

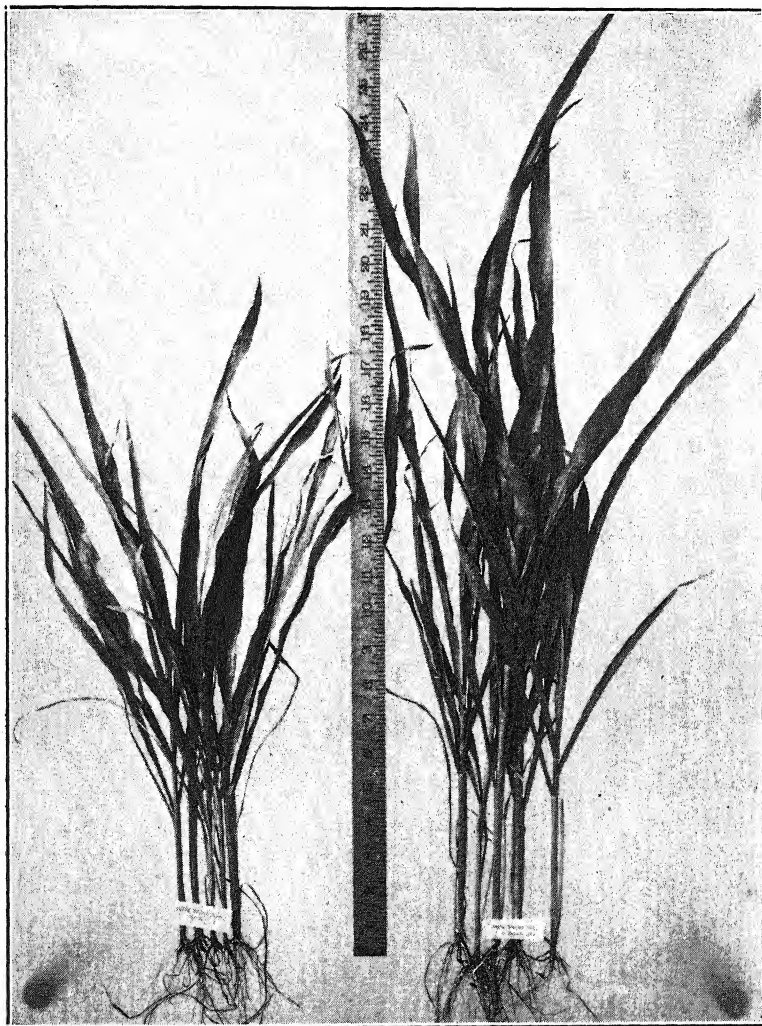


FIG. 1. Control of aphids and red spiders on Dwarf Yellow milo: Left, badly infested plants grown without selenium; right, noninfested plants grown in soil containing 2 p.p.m. of selenium.

the freedom from insect infestation persisted, although the controls became heavily infested.

Similar plantings also were made in soil containing 2, 3, and 4 p.p.m. of selenium. Measurements made 4 weeks after emergence showed no reduction in height of plants in the selenized soil. The controls, grown without selenium, soon became heavily infested with both aphids and red spiders. In the soil containing 2 p.p.m. selenium, red-spider infestation was observed after 8 weeks, but no injury was evident. The plants were green and healthy, while the controls without selenium were stunted and badly discolored (Fig. 1). After 14 weeks the plants grown in soil containing 2 p.p.m. selenium showed some evidence of red-spider reproduction and injury. Those grown in soil containing 3 p.p.m. selenium showed some infestation but no apparent injury, while those in soil containing 4 p.p.m. selenium remained free from red spiders and aphids. The *Leoti sorgo* grown in the selenized soil had formed normal heads and seed after 14 weeks, while, in the control soil, the plants were stunted and had produced no heads.

The length of time that one application of selenium to the soil will provide protection against aphid and red-spider infestation, and the optimum time, manner, and rate of applying additional selenium remain to be determined. It remains to be determined also what effect, if any, the selenium may have on the development of the several diseases of sorghum. It was noted that sorghum root rot developed as abundantly in the selenized as in the nonselenized soil, which indicated that the selenium did not inhibit the development of this disease.

It should be emphasized that selenium is extremely poisonous to man and other warm-blooded animals and, therefore, under no circumstances should it be used as here described in connection with plants intended for other than experimental purposes.—R. W. LEUKEL, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Sectoring in Colonies of Aplanobacter stewarti.—For the purpose of stimulating dissociation, 5 single-cell cultures of *Aplanobacter stewarti* have been used during the past year in daily serial transfers to different broth combinations and reactions, and daily platings have been made on beef-peptone agar. These 5 cultures produce wilt lesions on corn and develop on beef-peptone agar as typical yellow colonies, but they differ more or less in pathogenicity and in physiological reactions. After alternating periods of daily transfer and of ageing, no dissociation was observed on beef agar; but, on potato-dextrose-agar slants of culture No. 3b6, pure white areas developed in the yellow growth. Platings on potato-dextrose agar from the same broth cultures gave numbers of colonies nearly all of which were sectoring for color and possibly other characters. On 1 plate there were 3 colonies, 2 of which had lighter yellow sectors and 1 with a pure white

sector (Fig. 1). On the second plate were 50 colonies, a few pure yellow and a few pure white without sectors; but most of the colonies had 1 or more sectors of different shades of yellow or white. Three colonies on the first plate and 20 on the second were selected for further study. Transfers were made from each colony and from 1 or more sectors in that colony, making 53 transfers. Each of these transfers was replated for purity on potato-dextrose and beef-extract agar.

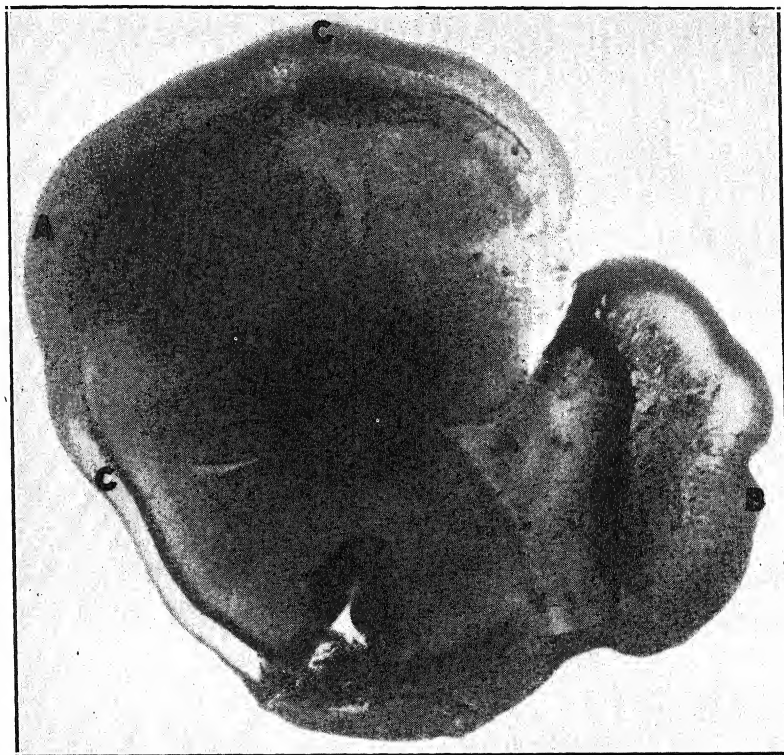


FIG. 1. Sectoring colony of *Aplanobacter stewarti* 38A3b6 (single-cell isolation). Plate I, colony 1. On potato-dextrose agar. Yellow colony with pure white sector. Photographed on April 17, 1939. A. Yellow colony. B. Pure white sector. C. White streaks. $\times 4$.

From 2 pure white colonies without sectors, only white colonies were obtained. From 9 transfers from white colonies without sectors, white colonies with yellow sectors and white sectors in yellow colonies, all but 2 gave pure white cultures. One transfer from a white colony without any apparent sectors gave a few yellow in many white colonies. One transfer from a white sector in a yellow colony gave 1 yellow in many white colonies. Platings from 4 light yellow (almost white) colonies or light yellow (almost white) sectors gave either light yellow colonies, white and yellow, or yellow colonies. Four yellow colonies, which had white sectors, gave only yellow colonies. Thirty platings from yellow sectors in light yellow colonies, yel-

low colonies, with light yellow sectors, yellow sectors in yellow colonies, and yellow colonies with yellow sectors gave white colonies in only 2 plates. Pure yellow colonies without sectors gave only yellow colonies. Thus some white colonies remained white, most yellow remained yellow, some light yellow segregated into white and yellow, while others remained light yellow or resumed the typical yellow of this species.

When inoculated into corn and reisolated from wilt lesions, white cultures remained white and yellow came out yellow.

The parent single-cell culture No. 3b6 is only weakly virulent, while the other 4 single-cell cultures are virulent. In 4 tests for pathogenicity with the 53 transfers of white and yellow colonies or sectors, there was no evidence that any of these cultures from sectoring colonies of 3b6 was more virulent than the original culture.

The 3b6 parent culture also differs from the other 4 single-cell cultures in that the reaction in litmus milk is sufficiently alkaline to produce a blue color, which darkens as the culture ages. All of the other 4 cultures are slightly acid in litmus milk, producing a pink color without curdling the milk. This is the typical reaction for *Aplanobacter stewarti*. The 53 transfers from white and yellow colonies and sectors when grown in litmus milk all produce the blue color typical of the parent strain 3b6. Variation in this instance appears to be for color only, the organism remaining stable as far as other characters tested are concerned.—CHARLOTTE ELLIOTT and ALICE L. ROBERT, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

A Specimen-envelope Folder.—Many laboratories and herbariums use specimen envelopes. When these are made by the usual technician or laboratory assistant they may vary considerably in size and shape.

A mechanical device that gives uniformity of envelope has been in use in our laboratory and is here illustrated and described. It is suited for making envelopes $3\frac{3}{4} \times 5\frac{1}{4}$ in. from 8×9 in. paper. The same general pattern can be modified to make envelopes of any desired size. The apparatus is of simple and easy construction and was designed by the late D. S. Giddings of our laboratory.

The device consists of a firm back, 10×11 in., of fiber board or thin wood on which guide blocks are to be fastened, and two iron plate folders. A white covering of paper or thin cardboard is to be fastened on the back. It is best first to make a drawing showing where the wood blocks are to be fastened.

To this flat back, thin blocks of wood are nailed. In our folding device these blocks were cut from the rounded ends of 12-in. pot labels $1\frac{1}{8}$ in. wide. Three different lengths of these blocks are required: 4 blocks $2\frac{1}{2}$ inches long (Fig. 1, A); 2 blocks $2\frac{3}{8}$ inches long (Fig. 1, B); 2 blocks $1\frac{1}{2}$ inches long (Fig. 1, C).

In placing the blocks A, B, and C, extra space of $\frac{1}{16}$ in. should be allowed

for all distances between blocks. This will provide room for operating the folders and placing the paper.

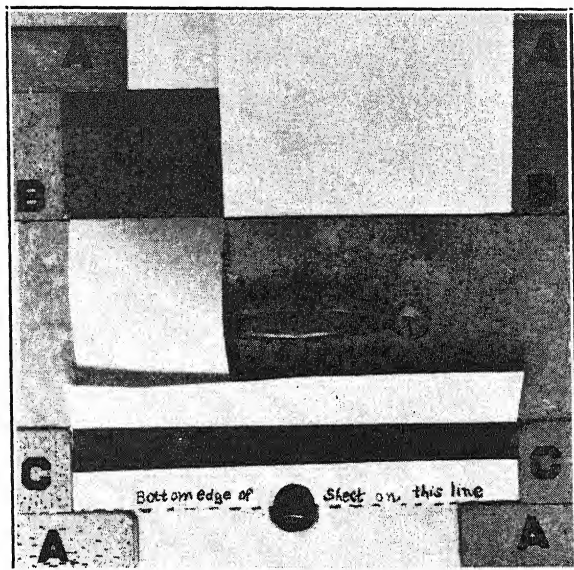


FIG. 1. General view of specimen envelope folder: A, B, and C represent blocks and show their relation to the iron plate folder, to the paper, and to the first folds of the envelope. The upper fold is cut to show the position of the upper blocks, A and B.

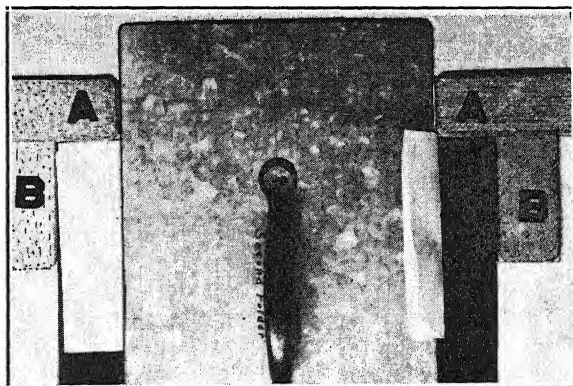


FIG. 2. A and B show blocks in position on back board and their relation to second iron plate folder and envelope, one end of which is already folded.

The two rectangular iron folder plates are shown in place in figures 1 and 2. They are made from thin galvanized iron and are each provided with a handle. The long edges of the plates are ground on the upper side to form an angled edge to give the folded paper a sharp crease. They are each 10 in. long, the same size as the width of the apparatus. The narrow folder is $3\frac{3}{4}$ in. wide, the width of the envelope to be made. Figure 1 shows

this folder in position where it is to assist in the making of 2 folds in the paper. The lower fold is already made. The top of one part of the envelope paper is in position unfolded (Fig. 1), while, at left, the portion is folded in the manner that the uncut sheet would be in practice. The second or wide plate folder for making the 2 end folds of the finished envelope, is $5\frac{1}{4}$ in. wide and equals the length of the folded envelope. Figure 2 shows it in position and one end of envelope already folded. It is more convenient to make folds in a number of envelopes with the first folder before completing the end folds with the second.—CLAYTON O. SMITH, University of California, Citrus Experiment Station, Riverside, California.

The Chilean Tomato, Lycopersicon chilense, Found Resistant to Curly Top.—In the February issue of *Phytopathology* for 1939, F. O. Holmes¹ published a note on the Chilean tomato, *Lycopersicon chilense* Dun., as a possible source of disease resistance. Through his kindness cuttings of this species were obtained for the purpose of testing their reaction to curly top. They grew well, and it was possible to get many plants from them.

The first test was conducted in the University greenhouse at Moscow, Idaho. Beet leaf hoppers (*Eutettix tenellus* Baker), which had been fed on curly-top sugar beets, were placed on 2 *Lycopersicon chilense* plants and on 2 tomato plants of the variety Earliana. Cages were placed over the plants to keep the insects confined to them. The leaf hoppers were left for 48 hours before their removal. The two Earliana tomato plants became severely diseased with curly top and died, whereas the *L. chilense* plants remained healthy, and no symptoms of the disease were observed.

The second test was conducted in the field at Buhl, Idaho, where curly top is usually severe every year. Small *Lycopersicon chilense* plants, started in the greenhouse from cuttings, were transplanted to the field. After they had established themselves beet leaf hoppers were caged on 3 of the plants. These leaf hoppers had been fed on diseased sugar beets prior to placing them on the *L. chilense* plants. The cages were removed after several days. No symptoms of curly top developed, and the plants grew luxuriantly throughout the season. No natural infection occurred on them, as did on the common tomatoes growing in the same field.

It appears that *Lycopersicon chilense* is resistant to curly top, and an attempt is being made to incorporate this resistance in several varieties of the common tomato.—WALTER J. VIRGIN, University of Idaho, Moscow, Idaho.

¹ Holmes, F. O. The Chilean tomato, *Lycopersicon chilense*, as a possible source of disease resistance. *Phytopath.* 29: 215-216. 1939.

EVIDENCE FOR THE IDENTITY OF THE YELLOW-SPOT VIRUS WITH THE SPOTTED-WILT VIRUS: EXPERIMENTS WITH THE VECTOR, THRIPS TABACI^{1,2}

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In June, 1937, an outbreak of a tomato disease, indistinguishable from spotted wilt, was observed by G. K. Parris of the Hawaii Agricultural Experiment Station, in a small field on the island of Oahu, Hawaii (31). To identify the causal virus and to obtain evidence for the coidentity of the tomato spotted-wilt virus with the pineapple yellow-spot virus,³ which has long been established in Hawaii, a project was started jointly by the Hawaii Agricultural Experiment Station and the Pineapple Experiment Station. Parris undertook symptomatological studies and mechanical transmission tests, and his data are presented in a concurrent paper (32). The writer conducted experiments on insect transmission of Y.S. virus to tomato and various other plants. Data are presented here showing that Y.S. virus is the causal agent of the tomato disease and that the host range and symptoms of the two viruses are similar.

REVIEW OF LITERATURE

Brittlebank (9) recorded the first appearance, in 1915, of tomato spotted wilt in Victoria, Australia. Pittman (33), Samuel *et al.* (37), and Bald *et al.* (5) in 1927, 1930, and 1931 demonstrated that the virus is transmissible by *Thrips tabaci* Lind. and *Frankliniella lycopersici* And. (3) and also by the rubbing method of mechanical inoculation with expressed plant juice. The specific relationships between the virus and vectors, longevity, and heat resistance of the virus *in vitro*, and many host plants were reported.

Smith (41, 42), in 1931 and 1932, discovered the same virus in England, where *Thrips tabaci* is the sole vector, and reported on the filterability of the virus and its host plants. Furthermore, Moore (30) in 1933 discussed the possible coidentity of Kromnek disease in South Africa, which is transmissible by *Frankliniella* sp., with spotted wilt. Her final conclusion on this and reference to the specific vectors, *F. schultzei* (Trybom) and *T. tabaci*,⁴ by E. E. Anderssen, both of which will be published shortly, were cited by Carter (13). Since 1929 the same virus has been discovered in California, Wisconsin, and Ontario (7, 16, 22, 40). Gardner *et al.* (18, 19, 20) in 1934, 1935, and 1937 reported from California its transmission by *T. tabaci* and

¹ Published with approval of the Director as Technical Paper No. 129 of the Pineapple Experiment Station, University of Hawaii.

² This investigation was conducted under the general direction of Dr. Walter Carter.

³ Hereafter the spotted-wilt virus will be referred to as S.W. virus; the yellow-spot virus as Y.S. virus.

⁴ The addition of *T. tabaci* is, with his permission, directly cited from an oral communication.

Frankliniella moultoni Hood⁵ together with a long list of host plants. McWhorter and Milbrath (27, 29) stated that the virus of tomato tipblight, known in southern Oregon since 1931, is very closely related but distinct from S.W. virus, although it is transmitted by *T. tabaci* also.⁶

In addition to the above references, there are more than 100 papers dealing with economic significance, new invasion or distribution, and host range in Australia, Europe, Africa, and North America. India is also included in the distribution data (26). The known host range is very large and a total of 101 host plants was enumerated by Smith (45) in his latest review. This number of hosts of a single virus is exceeded only by those of curly-top, aster yellows, tobacco mosaic, and cucumber mosaic. Many other valuable contributions have been recently published concerning chemical and physical properties and serological response of the virus, epidemiology of the disease, and environmental effects on symptom manifestation.

Linford (25) in 1932 published an account of pineapple yellow spot, which had been known in Hawaii since 1926. The virus was demonstrated to be transmissible by *T. tabaci*. The specific relationships between the virus and vector were found similar to those of S.W. virus. Preliminary experiments on mechanical transmission to pineapple were reported as being negative. Later, Carter (11) reported a single successful transmission by the needle puncture method. In addition to pineapple and *Emilia sonchifolia* DC.,⁷ a major weed host of the virus, Linford (24) added pea to the host list. He also prepared in 1931 a manuscript⁸, which still remains unpublished, but citation of which was granted, on the host range and symptoms. In the manuscript he mentioned that the virus was experimentally transmitted by *T. tabaci* to 17 other species of plants (his complete list has been cited in another paper (35)), including pepper, tomato, tobacco, and eggplant. Symptoms on some plants were described in detail and on others, very briefly Kitamura⁹ mentioned 6 susceptible plants including one addition to the host list.

Linford recognized the close similarity between Y.S. and S.W. viruses with respect to the vector and the specific relationships between the vector and virus (25), but only a partial similarity with respect to the symptoms

⁵ Inclusion of *F. occidentalis* Perg. as the vector (17), according to a private correspondence from Dr. S. F. Bailey, University of California, is a matter of different view on classification of the species. Permission was granted to cite his unpublished data.

⁶ *T. tabaci* was reported in a recent paper (Chamberlain, E. E., and G. G. Taylor. N. Z. Jour. Sci. and Tech. 20: 133A-142A. 1938) to be the vector of S.W. virus in New Zealand.

Dr. A. S. Costa, Instituto Agronomico do Estado de S. Paulo, Campinas, Brasil, recently informed the writer that the virus of "Vira-cabeca" of tobacco and tomato, widely distributed in Brasil, has been proven to be identical with S.W. virus; that the vector is a species of *Frankliniella*, possibly *F. paucispinosa* Moulton, which is also known to be the vector of "Coreova" of tobacco and tomato in Argentina. (Fawcett, G. L. Est. Exp. Agr. Tucuman Cir. 60. 1938). Identity of "Coreova" with "Vira-cabeca" and spotted wilt has not been reported.

⁷ Hereafter *Emilia sonchifolia* DC. will be referred to as *Emilia*.

⁸ Linford, M. B. Some hosts and symptoms of pineapple yellow spot.

⁹ Kitamura, F. T. The influence of host sequence on the efficiency of *Thrips tabaci* Lind. as a vector of the yellow-spot virus of pineapple. Thesis, Univ. of Hawaii. 1936.

and host range.¹⁰ However, he was unable to come to any conclusion as to their coidentity from his data. Smith (42, 45) speculated on the probable identity of the two viruses. Although their coidentity has thus been long postulated, it has still remained to be verified with further proofs. Recently, the following new data further suggesting their coidentity have been reported. Gardner *et al.* (19) and, later, Whipple (49) reported transmissions of S.W. virus to *Emilia*, and Whipple stated that the symptoms were almost identical with those of Y.S. virus. Linford (24) stated that the symptoms of Y.S. virus on pea were similar to streak in the continental United States, and, later, Whipple (49) and Snyder *et al.* (46) transmitted S.W. virus to garden and sweet peas and concluded that this virus is a causal agent of streak. It is induced from the above that the symptoms of the two viruses on this host must be similar. Lewcock (23) and Carter (13) observed a pineapple disease identical with yellow spot in Queensland and South Africa, where S.W. virus was well established. Although neither of them presented any experimental data, there is little doubt that the causal agent is S.W. virus.

Finally, Parris (32) demonstrated that Y.S. virus was readily transmitted to tomato, *Emilia*, and potato by the carborundum method of mechanical inoculation, and this first accomplishment of mass mechanical transmission contributed an important direct evidence for the coidentity of the two viruses. He concluded that the tomato disease observed in Hawaii is caused by Y.S. virus.

FIELD OBSERVATIONS

Emilia plants were found growing abundantly in the tomato field, where the outbreak of the disease was observed. Random samples of these *Emilia* plants showed very high populations of *Thrips tabaci* (6.81 per plant) and incidence of virus infection (79.8 per cent) among them, whereas populations of *T. tabaci* on tomatoes were negligible (0.23 per sample of twigs collected). The symptoms on tomatoes were indistinguishable from those of spotted wilt and the symptoms on *Emilia* from those of yellow spot.

Subsequently, the field *Thrips tabaci* from these *Emilia* samples were transferred to 12 each of noninfected *Emilia* and pineapple seedlings. Ten each of both plants were infected, showing the presence of a very high percentage of viruliferous insects in the field. The symptoms appearing on *Emilia* and pineapple were identical with those of yellow spot. These field observations and preliminary experiments suggested that the virus present in the tomato field was Y.S. virus, that the infection of tomatoes was also due to the same virus, and that the symptoms of Y.S. virus on tomato were similar to those of S.W. virus.

MATERIALS AND METHODS

Insects. Noninfective stock colonies of *Thrips tabaci* were established from adults collected on field onions which are nonsusceptible to Y.S. virus,¹¹

¹⁰ See footnote 8.

¹¹ See footnote 9.

and were maintained on *Emilia* or onions throughout the experiments. The noninfectiveness of such colonies was frequently checked.

Virus. *Emilia* infected in the field by Y.S. virus was taken as the original source of the virus. The infective colonies of thrips were established on such plants by transferring insects from the noninfective colonies. Infected *Emilia* and tomatoes collected in the tomato field, where the outbreak of the disease was observed, were also employed.

Plants. *Emilia* was constantly used as the indicator plant, and pineapple seedlings also were used in the experiments with tomato. Tomato and 21 other species of plants were used in the host range experiments. The variety of tomato used throughout the experiments, excepting those specified in the varietal experiments, was Marglobe.

Noninfected plants were grown from seeds, except *Emilia*, which was freely propagated by cuttings, and were used while they were young. They were planted singly in tin cans and covered individually by various types of insect-proof cages that confined the whole plant. A tall cylindrical celluloid cage, 3 by 18 inches, with 6 large cloth-covered windows, was found satisfactory for young tomato seedlings. The plants were kept in a greenhouse with open sides.

Methods. Methods of handling the vector differed from those employed by former workers (25, 37, 42). Insects to be transferred were shaken off from source plants onto black paper and sucked into medium-size reservoir vials, from which random individuals were then sucked into small vials, 15 by 25 mm., that were inserted directly into the cages containing the test plant. In most cases 5 insects were transferred per plant. For localized feeding, the felt-cage method (34) was employed. These operations were performed in small laboratory rooms, which were thoroughly sprayed after every unit of transfer. Other routine precautions were taken to avoid confusion of insects from different sources.

It is known that the virus is not retained through the egg stage; the adults are unable to become infective *de novo*; and the incubation period within the vector usually lasts until after the insect's emergence (5, 25, 42). Therefore, for acquiring or recovering the virus from infected source plants, noninfective young larvae were transferred and allowed to feed on such plants during their entire larval period. For transmitting the virus, freshly emerged adults from infected source plants were transferred to test plants.

TRANSMISSION OF THE YELLOW SPOT VIRUS TO TOMATO

The first experiment was to produce the symptoms of Y.S. virus on tomato. Sources of the virus were twofold, one from the yellow-spot-infected *Emilia*, and the other from the infected *Emilia* collected in the tomato field where the outbreak of the disease was observed. Young larvae from a noninfective stock colony were transferred to these plants, and adults emerging from these respective colonies were transferred to *Emilia*, pineapple, and tomato test plants (Table 1). Tomato, even when young, was found to be an unsuitable

TABLE 1.—Transmission of the virus, by *Thrips tabaci*, from yellow-spot-infected *Emilia* and infected *Emilia* collected in the tomato field where the outbreak of the disease was observed

Source of virus	Test plants	No. of test plants			
		From <i>Emilia</i> to			Transmission back to <i>Emilia</i> from tomato
		<i>Emilia</i>	Pineapple	Tomato	
Yellow-spot-infected <i>Emilia</i>	Tested	10	10	10	40
	Infected	6	2	5	1
Infected <i>Emilia</i> from the tomato field	Tested	10	10	10	51
	Infected	10	9	7	16

food plant for *Thrips tabaci* under experimental conditions.

Cross transmission tests back to *Emilia* were all positive (Table 1). Freshly emerged adults from the colonies established on the experimentally infected tomatoes were transferred to *Emilia* test plants. The lower percentage of infection in the yellow-spot-infected *Emilia* group may be due to the aging of the infected source plants.

The symptoms produced by the virus from the 2 different sources were found to be indistinguishable on *Emilia*, pineapple, and tomato. This clearly indicates that the virus present among *Emilia* in the tomato field is none other than Y.S. virus, confirming the data of the preliminary experiment. The symptoms produced by Y.S. virus on tomato were found to be identical with those on the field-infected tomato and also with those of spotted wilt.

In addition to the above experimental infections, 70 out of 263 tomatoes of various varieties also were infected. The varieties used besides Marglobe were Break O'Day, Burbank, First Early, Globe, Ponderosa, Pritchard, Red Plum, Rutgers, Stone, and currant tomato (*Lycopersicum pimpinellifolium* Dunal). It was observed that all varieties were susceptible and that their symptoms did not vary significantly.

SYMPTOMS ON TOMATO

Symptoms on field-infected tomatoes and on plants experimentally infected by mechanical inoculation were fully described by Parris (32). Therefore, characteristic points observed by the writer on plants experimentally infected by means of the vector are briefly given as follows:

Primary lesions were not observed. Downward curling of leaves and inward rolling of leaflets appear first. Bronzing (Fig. 1, A)—round or irregularly shaped spots, single or concentric rings, zonation, network along veinlets, midrib or lateral vein banding, or large blotching—appears suddenly on young leaves and rapidly advances to dark grayish-brown necrosis with or without zonation (Fig. 1, B, C, D, E). Diffused chlorosis appears along margin of necrosis. Affected leaflets or leaves wither and drop with the advancement of necrosis on laminae and petioles. Bronzing, then necrosis in

a streak form appears on stems, especially at or near terminals causing an appearance of die-back (Fig. 1, F). Stem necrosis sometimes kills plants if infected when young. Growth is stunted but is later restored and lateral shoots appear. On these aged plants, leaf distortion and yellowish mosaic mottling appear together with occasional bronzing and necrotic symptoms. Concentric rings of water-soaked sub-epidermal tissue or of dark purplish-brown superficial pigmentation appear on green fruits, and concentric rings or diffused large round spots of orange yellow on a red ground on ripe fruits. When numerous, these ring spots coalesce. Some fruits on apparently infected plants fail to show any symptoms.

The infrequency of bronzing as a symptom of yellow spot compared with spotted wilt, first observed by Linford¹² and again by Parris (32) and the writer, seems to be due to the rapid advancement of necrosis over bronzed areas. The writer, however, is convinced that this difference can be entirely

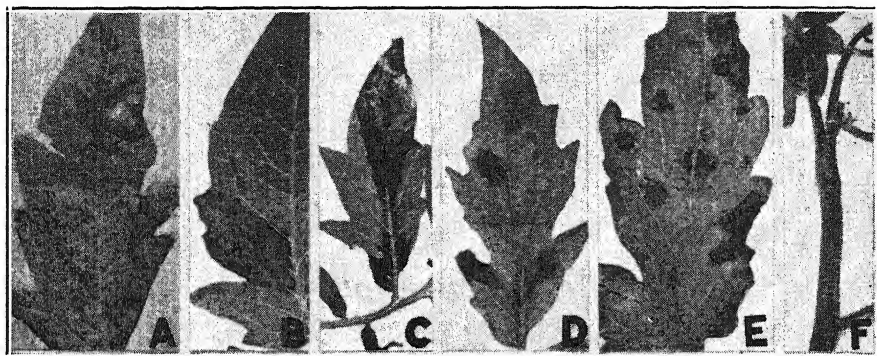


FIG. 1. Symptoms produced by the yellow-spot virus on tomato, experimentally transmitted by *Thrips tabaci*. A. Bronzing. B. Necrotic spot with concentric zonation. C-D. Necrotic midrib banding, blotching, and round spots with zonation. E. Necrotic round spots and rings, and veinlet necrosis. F. Necrosis on the petiole and stem at the tip.

attributed to the different climatic conditions under which the transmission occurred, but not to any difference between the two viruses. This opinion is based on the known facts that the symptoms of S.W. virus are variable under different climatic conditions (8, 18, 19, 30, 37, 38).

RECOVERY OF THE VIRUS FROM FIELD-INFECTED TOMATO

The next experiment was to recover the virus from field-infected tomatoes. Young larvae from noninfective stock colonies were transferred to the infected green or ripe fruits and to the twigs bearing fresh or aged symptoms, all of which were kept in large test tubes. The twigs bearing fresh symptoms from plants grafted with field-infected scions also were employed as source materials. Adults that emerged from these source materials were transferred to *Emilia*, pineapple, and tomato test plants (Table 2). A very high mortality of larvae on twigs of grown plants was observed. Cross transmission tests, with a similar procedure to that of the foregoing experiment, from the experi-

¹² See footnote 8.

mentally infected *Emilia* to tomato and also to *Emilia* and pineapple test plants were all positive (Table 2).

TABLE 2.—Transmission of the virus, by *Thrips tabaci*, from field-infected tomato

Source of virus	Test plants	No. of test plants					
		From tomato to			Transmission back from <i>Emilia</i> to		
		<i>Emilia</i>	Pine-apple	Tomato	<i>Emilia</i>	Pine-apple	Tomato
Fruits:							
Green	Tested	54	39	72	31	16	15
	Infected	14	6	3	25	15	8
Ripe	Tested	10	10	10			
	Infected	0	0	0			
Twigs:							
With fresh symptoms	Tested	10			15	5	5
	Infected	3			12	4	3
With aged symptoms	Tested	39	33	52			
	Infected	0	0	0			
Twigs from plants grafted with field-infected scions							
	Tested	57	35	35			
	Infected	11	8	1			

Similar to the findings with S.W. virus (1, 5), the virus was not recovered from ripe fruits and twigs with aged symptoms. The comparatively lower percentage of infection in this experiment seems to be attributable to unsuitableness of source plants for insect feeding and to loss of potency of the virus under certain conditions of the source plants. The symptoms produced on *Emilia*, pineapple, and tomato were indistinguishable from those of the experimentally infected plants by Y.S. virus in the foregoing experiment. These data on the recovery together with those on the transmission of Y.S. virus are adequate enough to conclude that the causal agent of the tomato disease in Hawaii was the long-established Y.S. virus, and not any other newly imported agent. Furthermore, it is definite that the symptoms of yellow spot on tomato are identical with those of spotted wilt.

HOST RANGE

Host range and symptom expression of a virus are taken as two of the criteria for its identification or classification. Here, the symptoms of Y.S. virus produced on various plants were directly compared with the published descriptions of the symptoms of S.W. virus. Twenty-one species of plants were tested, all but 5 of them crop plants. Nearly all of the crop plants of known status for the susceptibility to S.W. virus are included.

Experimental Procedures. Infective adults were transferred to test plants: The first lot, by freeing 5 adults directly into the cage; the second lot, by confining a single adult in the localized feeding felt cage (34), fastened on a young leaf for the purpose of detecting primary lesions; the third lot, by freeing 5 adults directly into the cages containing *Emilia* check plants in order to ascertain the infectivity of the insects (mostly, 10 plants for a single

unit of transfer). As a check for differentiating mechanical feeding injuries and symptoms, another lot of equal numbers of plants was similarly fed on by noninfective adults with both the free and localized feeding methods; non-infested check plants were kept in large numbers.

Recovery tests of the virus from the experimentally infected plants always were made, except on a very few plants on which larvae could not survive. Adults that emerged from the colonies established on the infected plants were retransferred to *Emilia* test plants. To ascertain the presence of masked symptoms or nonsusceptibility of plants, a similar transferring was made also from nonsymptom-bearing plants that, apparently, had been fed upon by infective insects. The carborundum method of mechanical inoculation was substituted in one case.

Numerical data are presented in table 3, with the combined figures of the infection by the free and localized feedings. The figures on the *Emilia* check lots, the noninfective adult check lots, and the recovery tests from infection-escaped plants of susceptibles are omitted. The degree of susceptibility for infection and suitability of the plants for insect feedings are summarized in the table.

Symptoms. The symptoms produced by Y.S. virus on broad bean, celery, potato, tobacco, *Nicotiana glutinosa* L., *Datura stramonium* L., petunia, and lettuce were found to be identical with those of S.W. virus. The characteristic symptoms on the respective plants are summarized in table 4. The susceptibility of *Datura stramonium* to Y.S. virus was first reported by Kitamura,¹³ but the symptoms he recognized did not agree with those of S.W. virus. Contrary to his findings, however, the symptoms observed in the present experiment were found to be fully identical.

Descriptions of the symptoms of spotted wilt on spinach, chicory, and endive, all susceptible (20), have not been heretofore published, making it impossible to compare with the symptoms of yellow spot; descriptions of the symptoms on eggplant and bell pepper are extremely brief (5, 41, 42) and critical comparisons also are impossible. However, the symptoms on the latter two produced by Y.S. virus were found to be of the same type as those described of S.W. virus. The symptoms on these 5 species of plants produced by Y.S. virus are described as follows.

Spinach. Primary lesions were not observed. Systemic symptoms appearing on heart leaves are scattered, small grayish-brown necrotic spots (Fig. 2, C) or dense massing of small brown necrotic specklings, marginal wilt, and necrotic streaks on petioles, all of which advance to kill leaves. Other associated symptoms are malformation, distortion, crinkling, marginal curling of leaves, and bending of petioles and midribs (Fig. 2, D). Growth is stunted. Necrosis advances very rapidly to outer old leaves and to roots, and then plants are killed in 10 to 20 days.

Eggplant. Primary lesions are at first large, diffused chlorotic spots ($\frac{1}{4}$ to $\frac{3}{8}$ inch in diameter) with a small, faint brownish center (Fig. 3, A), and

¹³ See footnote 9.

TABLE 3.—Transmission of the yellow-spot virus, by *Thrips tabaci*, from *Emilia* to various plants

Plants tested		No. of replica- tions	No. of test plants				Suscep- tibility to the virus	Feeding of <i>T. tabaci</i>	
			From <i>Emilia</i>		Transmission back to <i>Emilia</i>			Adult	Larva
			Tested	Infected	Tested	Infected			
<i>The susceptible group</i>	Viroflay	3	24	14	38	18	high “	good	good
	Spinach	1	12	9	20	11	“	“	“
	Broad bean	4	27	1	27	20	low	“	“
	Celery	2	7	5	28	15	high	“	“
	Potato	3	31	5	3	2	medium	“	“
	Eggplant	2	13	8	23	12	high “	“	“
	Bell Pepper	3	27	9	a		“	very poor	none
	Tobacco							poor	poor
	Tobacco	2	32	27	10	5	“	poor ^b	poor
	<i>Nicotinia glutinosa</i> ^c	2	36	26	12 ^{a,e}	2	“	very poor	none
<i>Datura stramonium</i>	Petunia	1	14	13	24	17	“	poor	good
		9	84	19	a		low	very poor	none
		2	14	9	41	28	medium	poor	good
Chicory	Endive	2	14	7	23	10	“	“	“
	Lettuce	2	18	6	20	9	“	“	“
<i>The non-susceptible group</i>									
<i>Commelina nudiflora</i> and <i>C. venghalensis</i>	Beet	1	16	0	40	0		“	“
	Swiss chard	3	15	0	12	0		poor	poor
	Spinach	3	18	0	34	0		good	good
Cabbage	Cauliflower	1	12	0	a			very poor	none
	Summer	1	8	0	6	0		poor	good
	chrysanthemum	2	20	0	17	0		good	“
	Unknown	1	8	0	11	0		“	“

^a No recovery tests were performed because larvae could not survive on the plants.^b Adults and larvae survived only on very young plants.^c Mechanical inoculation infected one each out of 6 *Emilia* and 6 *N. glutinosa*.^d Strains of Kelley and Lockwood were supplied by J. E. McMurtrey, Jr., U.S.D.A.^e Two strains from Sweden and Peru respectively were supplied by T. H. Goodspeed, University of California.

TABLE 4.—Summary of characteristic symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci* under greenhouse conditions

Plants	Primary lesions	Systemic symptoms			References on spotted wilt, with which the equations were compared
		On leaves	On stems and petioles	Effects on growth of plants	
ach		Necrotic spots (Fig. 2, C), marginal wilt, and distortion (Fig. 2, D)	Necrotic streaks	Lethal	
d bean	Leaves: Zonate necrotic spots (Fig. 2, A) Stems and petioles: Necrotic streaks (Fig. 2, B)	Zonate necrotic spots and marginal necrotic blotching	Necrotic streaks	Lethal	(42), (43)
y		Necrotic spots with chlorotic margin (Fig. 2, E)	Necrotic pockets (Fig. 2, E)	Retarded	(19), (20), (39)
to	Leaves: Zonate necrotic spots	Zonate necrotic spots (Fig. 2, F), and bronze rings and specklings	Necrotic streaks	Retarded	(28), (41), (42)
lant	Leaves: Concentric necrotic rings and zonate necrotic spots with chlorotic margin (Fig. 3, A, B)				(42)
pepper	Leaves: Zonate necrotic spots (Fig. 3, C)	Several types of mosaic mottling (Fig. 3, D, E)	a	Slightly retarded	(5), (41) (42)
ceo	Leaves: Concentric necrotic rings, zonate necrotic spots, and necrotic vein-outlining (Fig. 3, F-H)	Concentric necrotic rings, zonate necrotic spots, and necrotic vein-outlining (Fig. 3, I, J, K); mottling and distortion (Fig. 3, L)	Necrotic streaks	Stunted, sometimes lethal	(5), (30), (38), (41) (42), (47)
iana	Stems and petioles: necrotic streaks				
itnosa	Leaves: Zonate necrotic spots (Fig. 4, I) Stems and petioles: necrotic streaks	Zonate necrotic spots (Fig. 4, J), necrotic vein-banding, distortion, and yellowing (Fig. 4, K)	Necrotic streaks	Lethal	(5), (7), (30), (42)
ra		Zonate necrotic spots (Fig. 4, A), necrotic blotching (Fig. 4, B), concentric chlorotic rings, yellow vein-outlining, and distortion with yellow vein-banding and vein necrosis (Fig. 4, C, D)	Discoloration b	Stunted	(30), (41), (42)
amonium					
ia					
ry	Leaves: Zonate necrotic spots (Fig. 4, E)	Necrotic spots, necrotic blotching, distortion, and yellowing (Fig. 4, F-H)		Retarded	(7), (30), (42)
l endive		Necrotic spots, necrotic blotching, distortion, and yellowing		Retarded	(21), (28), (48)
ce					

Concentric chlorotic rings on fruits.
Malformation and necrosis on flowers and flower buds.

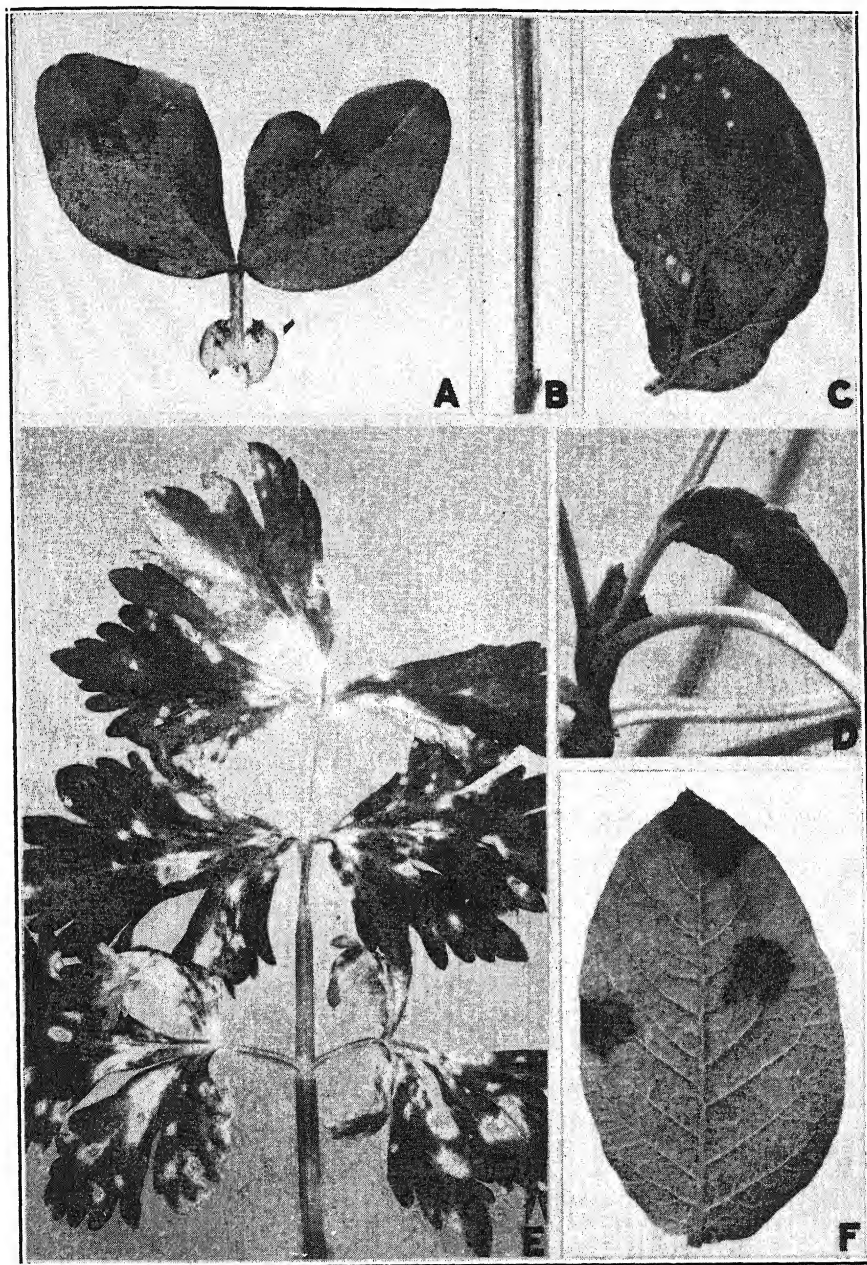


FIG. 2. Symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci*. A-B. Primary lesions on broad bean. Zonate necrotic spots on the laminae, and necrotic streak on the stem. C-D. Necrotic spots, marginal wilt, and abnormalities of the heart leaves of spinach (systemic). E. Necrotic spots with marginal chlorosis on the leaflets and necrotic pockets on the stem and petiole of celery (systemic). F. Necrotic spots with concentric zonation on potato (systemic).

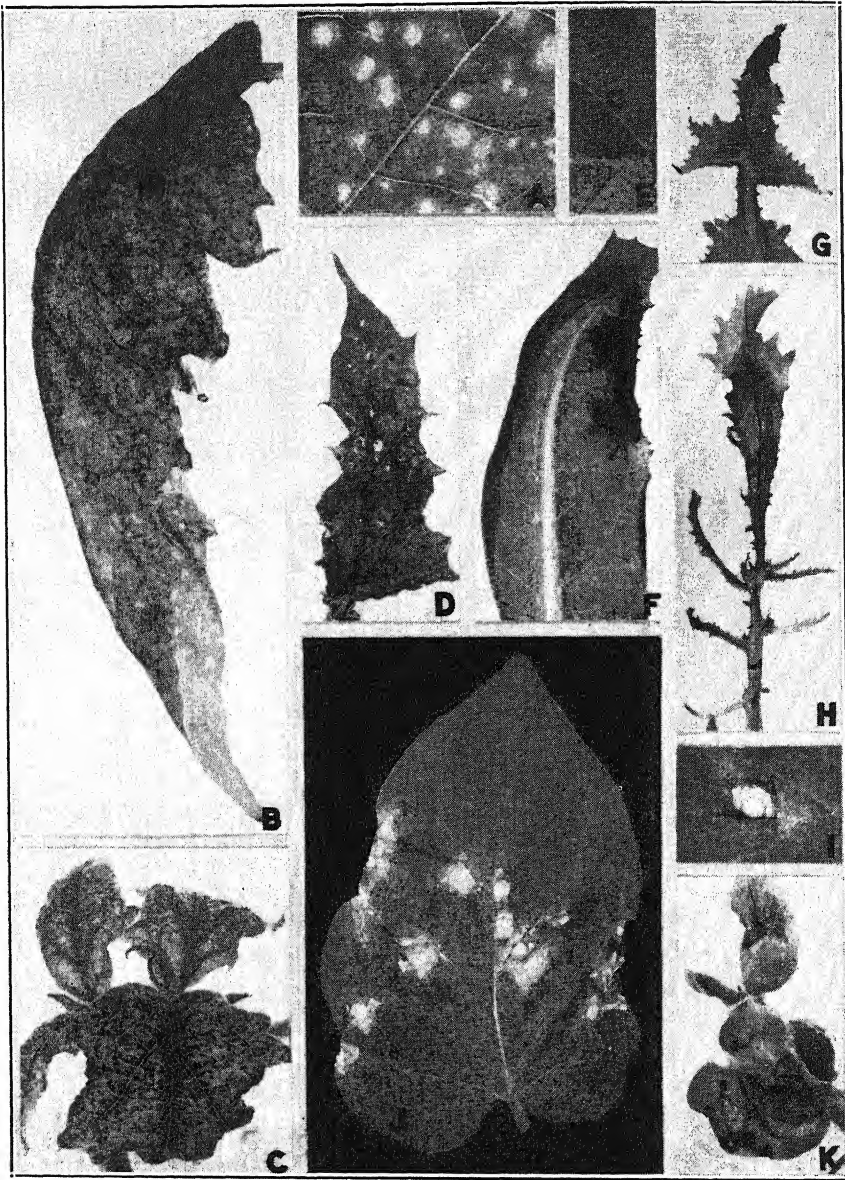


FIG. 4. Symptoms produced by the yellow-spot virus, experimentally transmitted by *Thrips tabaci*. A-D. Systemic symptoms on *Datura stramonium*. A-B. Pale ring spots advance to necrotic blotching with coalescence of necrotic spots; A, enlarged. C. Abnormalities of the terminal leaves, appearing on the recently infected plant. D. The same, appearing on the plant infected for some time. E. Primary lesions on petunia. Zonate necrotic round spots with darker margin (enlarged). F. Systemic symptoms on chicory. Necrotic blotching and curving. G-H. Systemic symptoms on endive. Necrotic blotching. I-K. *Nicotiana glutinosa*. I. Primary lesion. Zonate necrotic round spot with darker margin (enlarged). J-K. Systemic symptoms: J. Coalescence of zonate necrotic spots. K. Abnormalities of the terminal leaves.

No infection of S.W. virus on beet, chard, and cabbage (20), and New Zealand spinach (*Tetragonia expansa* Murr.) (41) was reported. Similarly, these plants were not infected by Y.S. virus.

In spite of the fact that cauliflower is susceptible to S.W. virus (19, 44), Y.S. virus was not transmitted to 20 plants under the writer's conditions. Infection of S.W. virus on chrysanthemum was reported (1, 44, 45). However, a closely related vegetable, summer chrysanthemum (*Chrysanthemum coronarium* L.), was not infected by Y.S. virus.

Field Infections. Field-infected plants of potato, bell pepper, and chrysanthemum, bearing indistinguishable symptoms of yellow spot or spotted wilt, were observed. These are, in addition to the 9 species of plants besides pineapple, pea, and *Emilia*, found by Linford to be naturally infected¹⁴ (35).

DISCUSSION

Former workers¹⁵ (24, 25) listed 21 susceptibles to Y.S. virus. The present experiments demonstrated that 14 species of plants were infected and 8 escaped infection. Nine out of 14 susceptibles have not been previously recorded.

Among these 30 susceptibles, 18 are known also to be susceptible to S.W. virus; but the susceptibilities of the rest have not been tested, since they are mostly local weeds. The susceptibles to both viruses are the 14 species of plants here reported and pineapple, pea, *Nicotiana glauca* Graham, aster, and *Emilia sonchifolia*. The foregoing experiments demonstrated that the symptoms produced by Y.S. virus on these 14 species of plants, except spinach, endive, and chicory, for which no published descriptions are available for comparison, are identical with those of S.W. virus. A pineapple disease, presumably caused by S.W. virus in Queensland and South Africa, was reported to be indistinguishable from yellow spot (13, 23). The identity of the symptoms of the two viruses on *Emilia* and pea was established (24, 46, 49). Linford¹⁴ stated that *N. glauca* and aster were infected by Y.S. virus and gave very brief descriptions of the symptoms. It is determined that his descriptions generally fit those of S.W. virus (5, 41, 42, 44).

Among the 8 species of plants that could not be infected, beet, chard, cabbage, and New Zealand spinach also are known to be nonsusceptible to S.W. virus (20, 41). The susceptibilities of two species of *Commelina* and summer chrysanthemum to S.W. virus are not known. In the entire present experiments, cauliflower was the only plant susceptible to S.W. virus and yet could not be infected by Y.S. virus. The writer, however, is convinced that the peculiarity of the conditions under which the experiment was performed was responsible for the failure of infection.

The general similarities of the host range and symptoms of the two viruses are thus well demonstrated, providing evidence for their coidentity.

The length of the latent period of a virus within a plant is specific for different viruses, and this character has been used by various workers to

¹⁴ See footnote 8.

¹⁵ See footnotes 8 and 9.

identify and classify a virus. The data on Y.S. virus obtained in the present experiments and also in the experiments of Linford (24, 25) are compared with those of S.W. virus compiled from the references (Table 6). Fair

TABLE 6.—Comparison of the lengths of the latent periods of infection of yellow spot and spotted wilt

Plants	Yellow-spot virus		Spotted-wilt virus		
	Primary lesions	Systemic symptoms	Primary lesions	Systemic symptoms	References
	<i>Days</i>	<i>Days</i>	<i>Days</i>	<i>Days</i>	
Spinach		10–20		not available	
Broad bean	5–6	10–12		not available	
Celery		30 ^a		not available	
Potato	8–10	20–30	14–16	25–28	(41, 42)
Eggplant	15		10		(42)
Bell pepper	15–20	30	21	not available	(41)
Tomato		8–27	7–8 ^b	7–21	(6, 15, 37, 42)
Tobacco	4–5	9–11	3–10	10–20	(5)
<i>Nicotiana glutinosa</i>	3–5	9–11	4–6	7–15	(5, 45)
<i>Datura stramonium</i>		10		8–10	(41)
Petunia	3–4		2–3		(42)
Chicory		15–20		not available	
Endive		15–20		not available	
Lettuce		15–25	10	10–25	(2, 48)
Pineapple	7–27 ^c		not available		
Pea	12–20 ^c		7–20		(49)
<i>Nicotiana glauca</i>	not available ^c		not available		
Aster	not available ^c		14–21		(42)
<i>Emilia</i>	8–27 ^c		not available		

^a Datum from a single plant.

^b The cases on very young plants were reported (6).

^c Cited from Linford¹⁶ (24, 25).

analogy on the majority of the plants is observed. This again is additional evidence for the coidentity of the two viruses.

The similarity of the mode of transmission of the two viruses has been demonstrated. S.W. virus is known to be transmitted by thrips vectors and mechanical inoculation. *Thrips tabaci* and 3 species of *Frankliniella* are vectors. *T. tabaci* transmits Y.S. virus but none of the above species of *Frankliniella* are distributed in Hawaii (35). Not only are both viruses transmitted by the same vector, but also the length of the incubation period within the vector and the specific stage of the vector for becoming infective are similar for both viruses (4, 5, 25, 30, 37, 42). The mechanical transmission of Y.S. virus, as easily accomplished as with S.W. virus, has been recently demonstrated by Parris (32). He also demonstrated that Y.S. virus is not seed-transmissible, similarly as in S.W. virus (10, 30, 37).

The similarities on the host range, symptoms, length of latent period within plants, and mode of transmission all suggest the coidentity of Y.S. virus with S.W. virus. The chemical and physical properties of S.W. virus

have been worked out with comparative thoroughness, but those of Y.S. virus are not known. However, since the technique of mechanical inoculation has now been established, these properties may be readily studied.

The outbreak of the tomato disease in Hawaii has been determined to be caused by the long-established Y.S. virus. Although this crop plant is most frequently and severely attacked by spotted wilt in all other areas, lower incidence of infection has been known in Hawaii. This may be attributed to the relative food preference of the insects, small acreage of tomato cultivation, and dissimilar distribution or centralization of the tomato cultivation and the viruliferous insect populations. However, this outbreak coincided with the disturbed host succession of the insects and planting of tomatoes within the infection center. The tomato field had been previously surrounded by potato patches that were harboring a high population of infected weeds and viruliferous insects. When these patches were plowed for the succeeding sugar-cane crop, the insects were compelled to disperse and concentrate in the nearby tomato field. There the insects were forced to feed on tomatoes, and, consequently, the outbreak suddenly appeared. A detailed study on migration of *Thrips tabaci* in relation to incidence of infection was published by Carter (14) and observation of a confirming incident also was reported by the writer (36).

SUMMARY

The yellow-spot virus was recovered by *Thrips tabaci* Lind. from field-infected tomatoes.

The yellow-spot virus was transmitted to and recovered from, by *T. tabaci*, spinach, broad bean, celery, potato, eggplant, bell pepper, tomato, tobacco, *Nicotiana glutinosa* L., *Datura stramonium* L., petunia, chicory, endive, and lettuce, which are known also to be susceptible to the spotted-wilt virus. The symptoms produced on these plants, except spinach, endive, and chicory, for which no published descriptions are available for comparison, were all identical with those of the spotted-wilt virus. The lengths of the latent period within the respective plants were also generally analogous. Beet, chard, cabbage, and New Zealand spinach, which are known to be not susceptible to the spotted-wilt virus, were also not infected by the yellow-spot virus.

These data provide clear evidence that yellow spot and spotted wilt are caused by the same virus.

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MECHANICAL TRANSMISSION OF YELLOW-SPOT VIRUS: EVIDENCE FOR IDENTITY WITH SPOTTED-WILT VIRUS¹

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In June, 1937, a diseased condition of tomato plants was observed at Waipahu, Oahu, Territory of Hawaii, characterized by necrosis of laminae and terminals, blotchiness of color and rugosity of surface of mature fruits, and a general cessation of growth (Figs. 1-3). Occasionally, slight bronzing of the upper surfaces of leaves was seen, prior to the appearance of necrotic lesions, and also bronzing of the stem ends of immature or partially mature fruits. A preliminary note recording the disease was published in 1938 (8).

The symptoms closely resembled those reported for spotted wilt on tomato with one exception, viz., bronzing was not outstanding. If one substitutes "necrosis" for "bronzing" in the descriptions of spotted wilt given by Samuel *et al.* (12), Bald and Samuel (1), and Smith (13), one has a fairly accurate and complete description of the disease as it appears in Hawaii, where it is common at present. Samuel *et al.* (12) have pointed out that bronzing is due to collapse and subsequent browning of the epidermal cells. Both of these terms signify necrosis. Furthermore, bronzing in Australia varies from an almost imperceptible glaze to so deep a bronze as to be almost black. It is faintest in glasshouse tomatoes or in very early field tomatoes, while in later field tomatoes *in the full summer sun*, discoloration is the darkest. These observations led Samuel *et al.* (12) to state, "This suggests some relationship between the development of bronzing and

¹ Published with the approval of the Director as Technical Paper No. 50 of the Hawaii Agricultural Experiment Station.

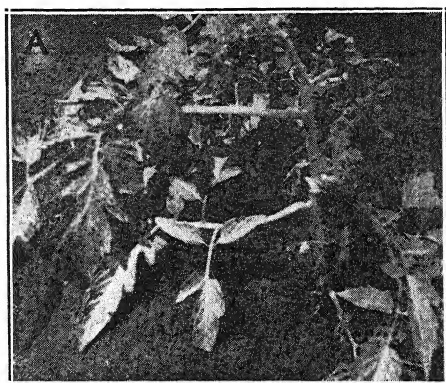


FIG. 1. Effect of yellow spot on tomato in field. A and B. Terminal necrosis, stunting of plant, twisting and curling of leaflets and petioles. C. Growing point of diseased plant. Note intralaminar spot necrosis and twisting of laterals, petioles, and leaflets. Dark streaks present on stem do not show to advantage. Photograph C by K. Sakimura.

the intensity of light (or heat)." The tomato disease in Hawaii appears to be spotted wilt with bronzing largely suppressed by environmental conditions.

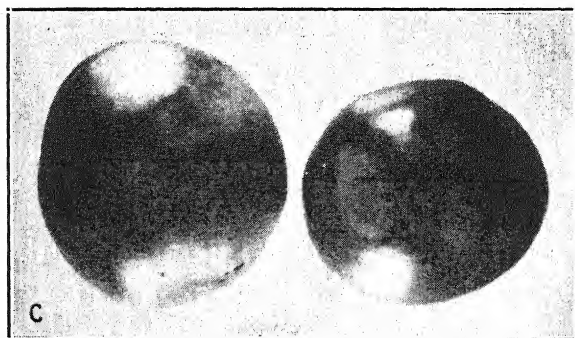
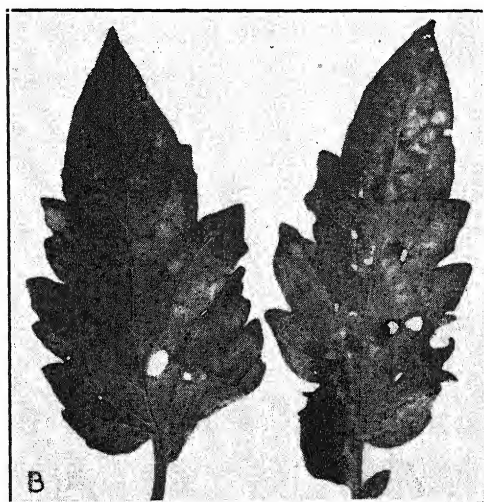
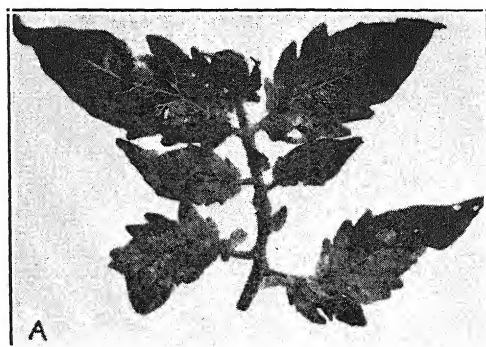


FIG. 2. Effect of yellow spot on tomato in field. A. Zonate banding pattern of necrosed tissue, accompanied by yellowing with little or no bronzing. B. More diffuse banding with increased yellowing. Severe necrosis at lower end of leaflet on right. C. Circular, yellowed areas, often zonately banded, in background of normal red surface of fruits. Photograph C by K. Sakimura.

M. B. Linford, plant pathologist of the Pineapple Experiment Station, Honolulu, T. H., first suggested to the writer that the local disease might be due to the virus of yellow spot of pineapple, for the symptoms closely resembled those obtained by Linford² on tomato (Fig. 4). The weed *Emilia sonchifolia* DC. (*sagittata*) had been reported by Linford (6) as a host of the yellow-spot virus, and examination of *Emilia*, growing in and around the tomato planting, where the disease was first observed, revealed that a large number of plants were diseased (Fig. 5).

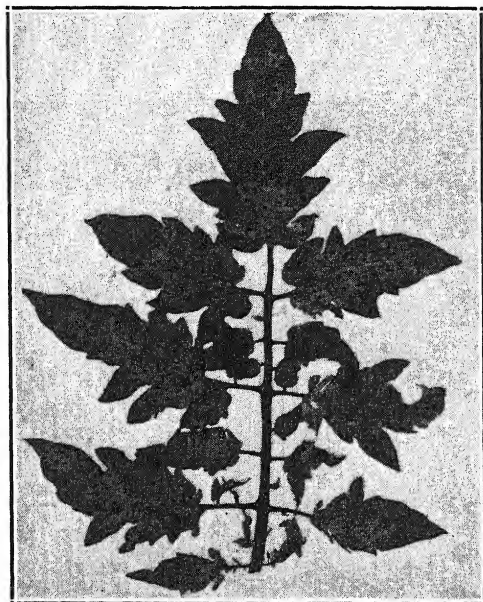


FIG. 3. Mottling or "mosaic" effect of terminal leaves of plants which have been diseased for some time, or whose growth has been slow. Note two small necrotic lesions at apex of terminal leaflet.

REVIEW OF LITERATURE

The yellow-spot disease of pineapple was shown by Linford (6) to be caused by a virus, transmitted by *Thrips tabaci* Lindeman. According to this investigator, G. E. Paxton failed to transmit the disease from pineapple to pineapple by mechanical means. Suspects listed by Linford were pineapple, *Emilia sonchifolia*, and unspecified members of the families Bromeliaceae, Liliaceae, Caryophyllaceae, Leguminosae, Labiatae, Solanaceae, Rubiaceae, and Compositae. Sakimura (10) has since published Linford's list of host species. Carter (2) successfully transmitted the virus by mechanical means from pineapple to pineapple in a single instance.

That the viruses of yellow spot and spotted wilt are due to a single entity has been suggested by several investigators. Smith (13) has pointed out that symptoms on *Emilia* are very similar, and, according to Dr. Walter

² Unpublished data: cited by Sakimura (10).

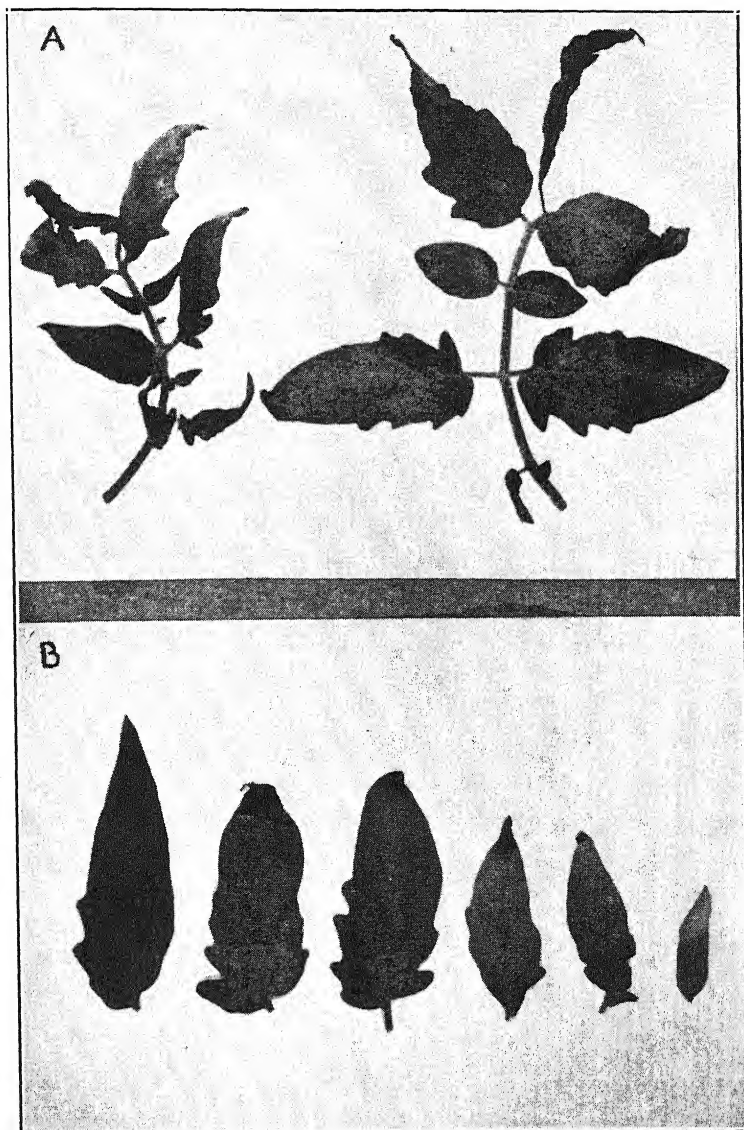


FIG. 4. Symptoms of yellow spot on tomato (var. Improved Stone) following inoculation, by *Thrips tabaci*, with the virus from *Emilia sonchifolia*. A. Distortion of leaflets and petioles and spot-necrosis and mottling of laminae. B. Necrosis of tips of leaflets and mottling of laminae. Healthy leaf at left. Photographs by M. B. Linford.

Carter,³ entomologist of the Pineapple Experiment Station, Honolulu, T. H., symptoms of yellow spot on *Emilia* are indistinguishable from symptoms of spotted wilt on *Emilia* in California. Gardner *et al.* (4) recorded spotted wilt on *Emilia* in California but published no photographs. Linford (5) transferred the virus of yellow spot from *Emilia* by means of *Thrips tabaci*

³ Oral communication.

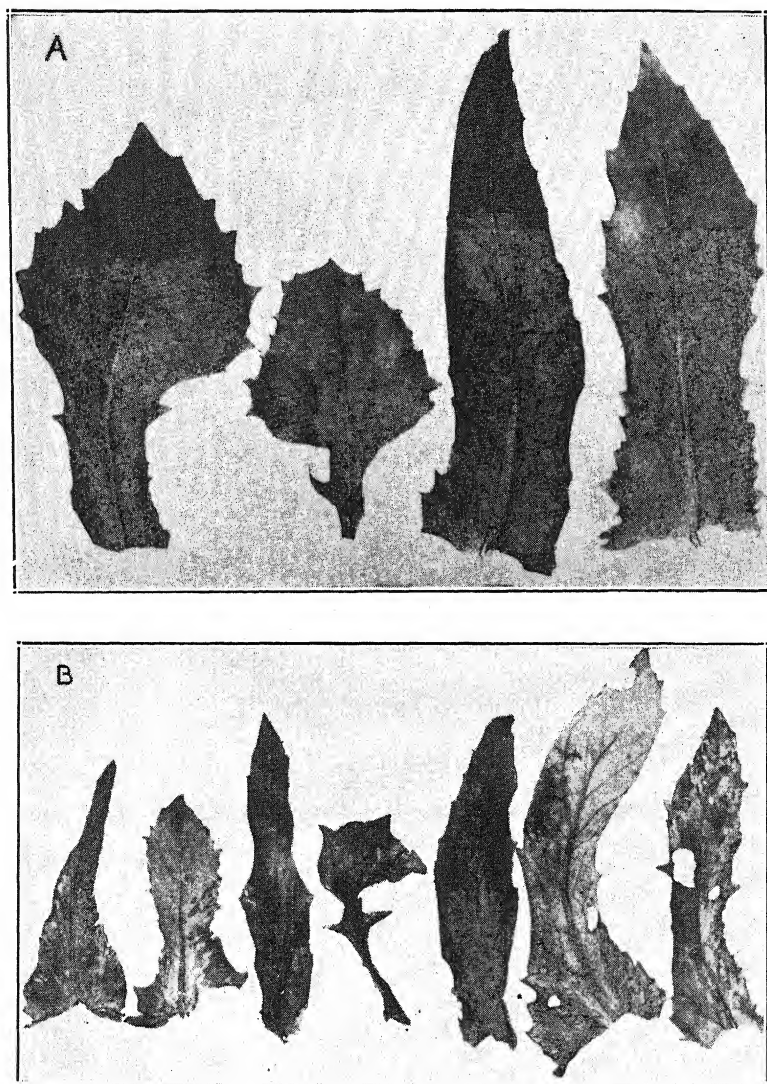


FIG. 5. Field symptoms of yellow spot on leaves of *Emilia sonchifolia*. A. Mottling and zonate banding with slight distortion. B. Pronounced chlorosis and distortion. Slight necrosis of third and fourth leaf from left.

to pea, not then recognized as a host of spotted wilt, to reproduce the symptoms of pea streak. He suggested that streak might be caused by the yellow-spot or a related virus. Later, Whipple (15) mechanically transmitted a virus from pea with streak symptoms, to tomato, *Emilia*, and other plants, and obtained symptoms identical with those caused by spotted wilt. Whipple pertinently states, "The demonstration that the tomato spotted-wilt virus causes a streak in pea is of peculiar interest inasmuch as the virus has much in common with that of the pineapple yellow spot in the

way of insect vector, incubation period, and the almost identical symptoms on *Emilia sagittata*." Snyder and Thomas (14) confirmed Whipple's work and conclusions.

The observations of Carter (3) on the association of yellow spot with *Kromnek* in the Union of South Africa, together with his citation of indications of identity of *Kromnek* and spotted wilt, obtained by Dr. E. S. Moore, further support the view that yellow spot and spotted wilt are caused by one virus.

This paper presents evidence that the local disease of tomato is caused by the same virus as yellow spot of pineapple.⁴

EXPERIMENTS ON TRANSMISSION OF THE VIRUS

1. *Seed*. Seeds collected from immature and mature diseased fruits were planted in soil in the greenhouse and examined daily after emergence for symptoms of the local disease. Fifty plants were grown to maturity, and 650 plants until the emergence of the fourth compound leaf. In no case did disease symptoms appear.

2. *Mechanical Inoculation with Expressed Plant Sap*. Sap was extracted from the tissues of plant parts of diseased tomato and of other diseased plants, i.e., *Emilia sonchifolia* and potato, by grinding with sterilized mortar and pestle and filtering through cheesecloth. This preparation was then rubbed on the leaves of healthy plants of tomato, *E. sonchifolia*, potato (var. Bliss Triumph), bell pepper (*Capsicum annuum*), garden pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), and lettuce (*Lactuca sativa*), with a glass rod. Carborundum powder was dusted on the leaves before abrasion as recommended by Rawlins and Tompkins (9). Three to 6 leaflets were inoculated when tomato was studied, and with other plants an equivalent area was treated. All plants inoculated were young and growing vigorously. For each series of inoculations an approximately equal number of plants was rubbed with juice from healthy plants and served as checks. Plants of both classes were grown side by side on the greenhouse bench, and none of the checks became infested.

The results of mechanical inoculations are given in table 1. The virus was transmitted mechanically from tomato to tomato and from tomato to potato, respectively, in 47 per cent and 75 per cent of the inoculations; from *Emilia* to *Emilia* and from *Emilia* to tomato, respectively, in 33 per cent and 24 per cent of the inoculations; and from diseased potato, following previous successful mechanical inoculation, to potato and tomato, respectively, in 57 per cent and 100 per cent of the inoculations. Negative results attended all attempts to transmit the virus by mechanical means from tomato to *Emilia*, lettuce, garden pea, or bell pepper. *Phaseolus vulgaris*, not a susceptible of the yellow-spot virus (10) and an uncertain susceptible of the spotted-wilt virus (13), also remained unaffected by mechanical inoculation.

⁴ Supporting evidence is being published concurrently (11).

Samuel *et al.* (12) report mechanical transmission of spotted-wilt virus from immature but not from mature diseased tomato fruit. In the present investigations, the local virus has been mechanically transmitted from immature tomato fruits in 5 out of 15 attempts. No transmission has been obtained with sap expressed from mature fruits with a similar number of inoculations.

The latent periods of infection of the virus in tomato, potato, and Emilia, respectively, are also shown in table 1. The younger and more vigorous the plant inoculated, the more rapid was the appearance of symptoms. This was particularly true of tomato.

Symptoms on tomato following mechanical inoculation (Fig. 6) corre-

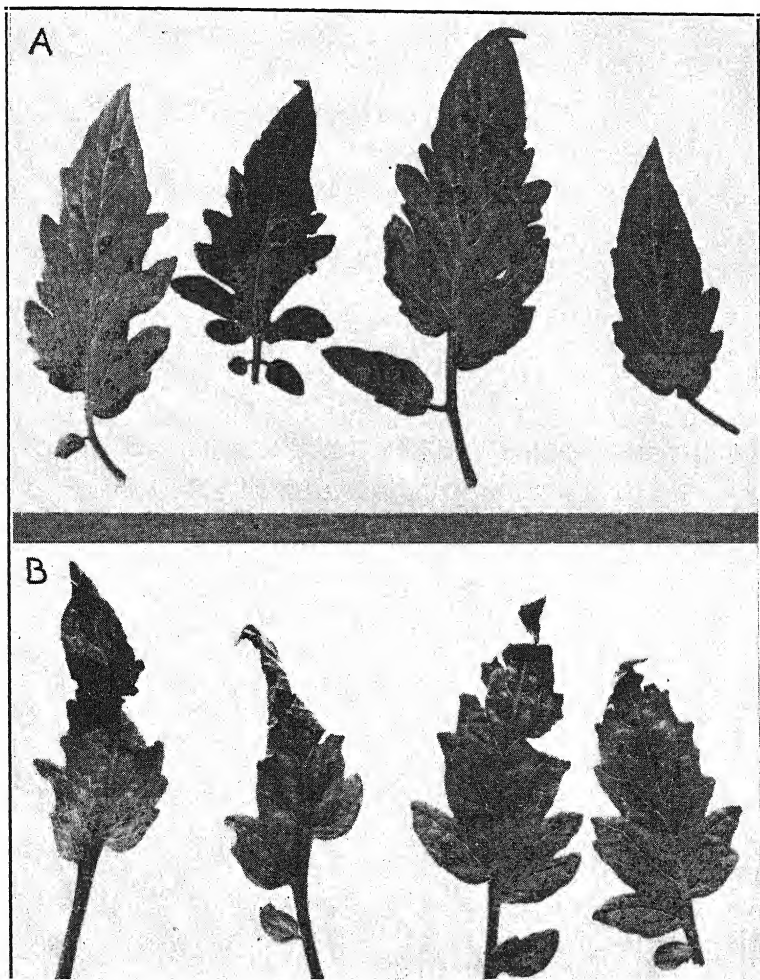


FIG. 6. Yellow spot on tomato leaves following mechanical inoculation. A. Necrotic lesions surrounded by yellow halo, with bronzing very slight or absent. B. Extreme necrosis and slight curling.

spond in intensity and type with conditions observed on plants naturally infected in the field and, in addition, are very similar to symptoms obtained by Linford with transmission by *Thrips tabaci*, previously mentioned and shown in figure 4. Necrosis of tips of tomato leaflets may accompany intralaminar lesions (Fig. 6, B). Fruits borne on diseased plants in greenhouse culture show the same type of blotchiness and rugosity of surface as exhibited in the field (Fig. 2, C). Mechanical inoculations are most successful with tomato when sap is extracted from the youngest tissues of recently diseased plants. It is very difficult to obtain positive results when sap is obtained from the old parts of diseased plants, or from any part of plants that have been diseased for some time or whose growth has been slow. The youngest parts of plants in this condition do not show typical symptoms: the foliage is mottled or "mosaiced," very much after the manner of typical tomato mosaic. Necrotic lesions are absent or of only minor significance (Fig. 3). Sap extracted from "mosaiced" leaves consistently gives negative results for the presence of a virus. This change in symptom expression and the failure to obtain a virus from "mosaiced" leaves are in accord with the findings of Samuel *et al.* (12) for spotted wilt. The transfer of "mosaiced" plants to fresh soil in larger containers causes the reappearance of the necrotic lesions on the leaves and petioles of the subsequently developed parts, and sap extracted from this new growth is highly infectious. Bald and Samuel (1) have noted that plants recently diseased are a much better source of virus than plants that have been diseased for a long time.

On the potato, systemic infection also occurs. One to 3 intralaminar, circular, concentrically zoned, necrotic spots appear on each leaflet; bronzing is slight or absent (Fig. 7, A). It is impossible to distinguish, without microscopic examination, between these spots and spots produced by the fungus *Alternaria solani* (E. and M.) Jones and Grout. Spores are usually present on fungous spots. In cases of severe infection the entire leaflet, or several leaflets and their petioles, may succumb (Fig. 7, B). Magee (7) has recorded the effect of the virus of spotted wilt of tomato on potato in Australia. Numerous circular, brown, dead areas are produced on the upper leaves of the plant, and longitudinal dead areas occur on the apices of the stems. The lesions are small and may show a characteristic type of zoning. Later, the spots on the leaves coalesce to form dead regions and the stems collapse at the apices. These symptoms closely approximate the effect of the virus of yellow spot of pineapple on potato.

On *Emilia*, the virus produces a distinct mottling or "mosaiced" effect of the young leaves, with subsequent development of circular, concentrically zoned spots on any portion of the laminae; these spots later may become necrotic (Fig. 8). Necrotic streaks may be produced on the petioles of diseased plants, and also on the peduncles and calyces of the inflorescences. Leaves may also be badly distorted and are often smaller than normal. These symptoms, with the exception of the necrotic spots, check fairly closely with

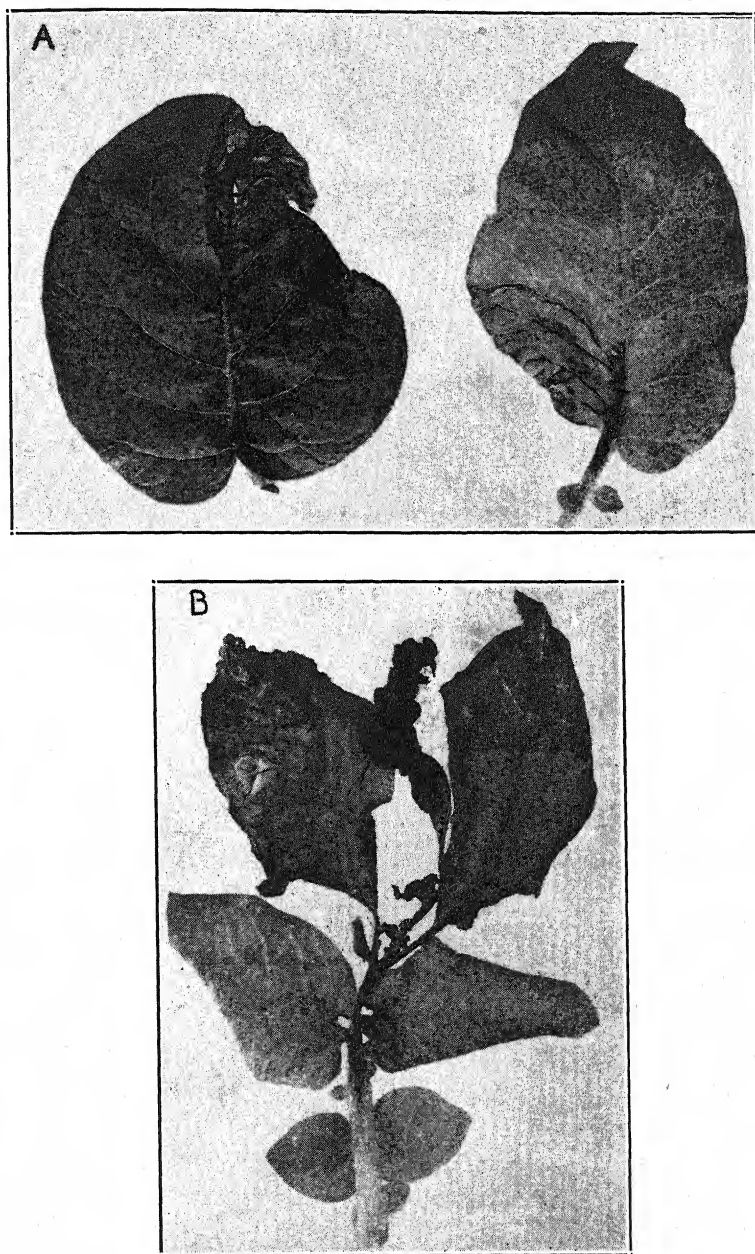


FIG. 7. Yellow spot on potato (var. Bliss Triumph) following mechanical inoculation. A. Necrotic lesions with zonate banding. B. Severe necrosis of several leaflets and portion of petiole. This type of reaction is seldom obtained.

the symptoms described on *Emilia* by Linford (6) for naturally diseased plants. This writer does not mention necrosis as a symptom of yellow spot on *Emilia*, but a careful search of diseased plants in the field has shown that

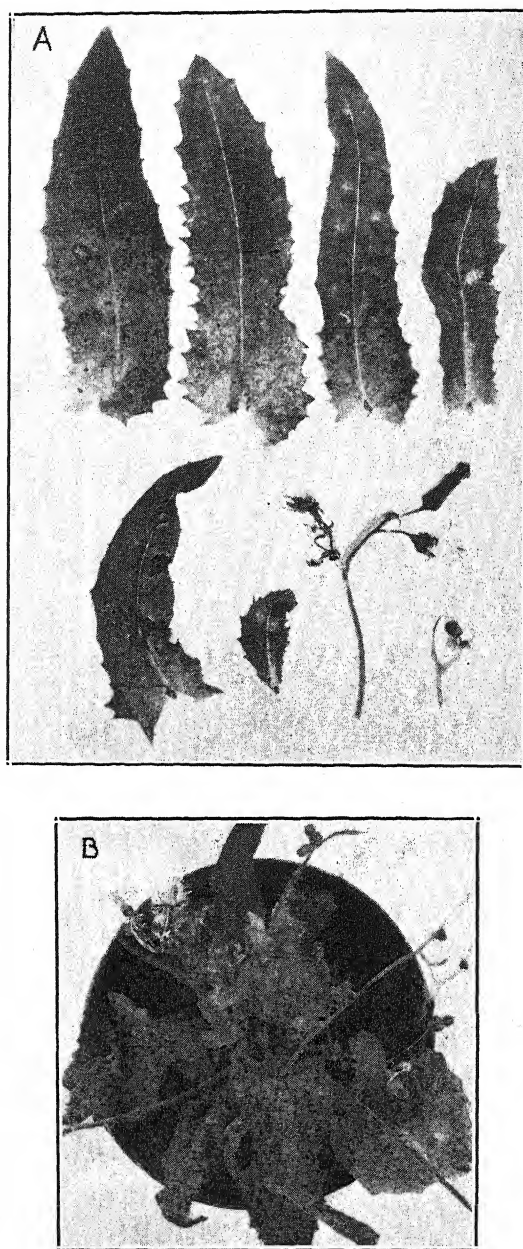


FIG. 8. Yellow spot on *Emilia sonchifolia* following mechanical inoculation. A. Zonately banded, chlorotic and necrotic spots on laminae, flower-pedicels, and calyces. B. Effect of virus on plant as a whole. Photographs by K. Sakimura.

occasionally necrosis may accompany the other, more typical, symptoms (Fig. 5, B).

3. *Grafting*. The virus has been successfully transmitted by grafting, as shown in table 2. Transmission occurred with all types of grafts tried,

TABLE 2.—Results obtained by grafting scions of tomato and potato, diseased with the virus of yellow spot of pineapple, into healthy tomato and potato, and by grafting healthy tomato into diseased tomato

Graft	Plants grafted	Plants infected	Latent period of infection	
			Time (days)	Number of plants
Naturally diseased tomato scion into healthy tomato	20	15	1-10 11-20 21-30	4 3 8
Mechanically inoculated diseased tomato scion into healthy tomato	15	12	1-10 11-20 21-30	3 3 6
Grafted diseased tomato scion into healthy tomato	2	2	11-20	2
Naturally diseased tomato scion into healthy potato	2	1	21-30	1
Mechanically inoculated diseased potato scion into healthy tomato	2	2	11-20	2
Healthy tomato scion into mechanically inoculated diseased tomato	1	1	11-20	1
Healthy tomato scion into grafted diseased tomato	3	3	11-20	3

infection ranging from 50 to 100 per cent. Symptoms on grafted plants are identical with symptoms observed on naturally infected plants in the field. The latent period of infection of the virus in grafted plants closely approximates that found with mechanically inoculated plants.

DISCUSSION

From the results obtained with mechanical inoculations and by grafting, it is concluded that the local disease of tomato is due to the virus of yellow spot of pineapple. Symptoms on tomato, *Emilia*, and potato are shown to be indistinguishable from symptoms reported for the effect of the spotted-wilt virus. Transmission studies by Sakimura (11) with *Thrips tabaci* show this to be true for other susceptibles. The spotted-wilt virus is transmitted mechanically and by grafting but is not seed transmitted. Data presented here show that the virus of yellow spot behaves similarly. These facts, together with the known relationship of pea streak to yellow spot (5) and to spotted wilt (14, 15), and of *Kromnek* to yellow spot and spotted wilt (3), points to a belief, expressed previously (13, 15), that the two viruses are identical, but physical properties have not yet been compared.

SUMMARY

1. A disease of tomato in Hawaii with symptoms identical with those of spotted wilt is described and evidence presented that the disease is due to the virus of yellow spot of pineapple.

2. The virus is not considered to be transmitted by seed, but is easily transmitted mechanically from tomato to tomato and potato, and from *Emilia sonchifolia* (*sagittata*) to *Emilia* and tomato. It has not been transmitted mechanically from tomato to *Emilia*, lettuce, garden pea, or bell pepper. The virus may also be transmitted by grafting, from tomato to tomato and potato and from potato to tomato. Symptoms artificially produced on tomato in greenhouse culture are identical with those found on naturally diseased field plants and are also similar to symptoms produced by Linford on tomato [unpublished: cited by Sakimura (10)] when the yellow-spot virus was introduced by the vector *Thrips tabaci*. Symptoms on potato are identical with those reported by Magee (7) for the virus of spotted wilt in Australia. On *Emilia*, the yellow-spot virus from tomato produces symptoms which closely resemble the effect of the virus on *Emilia* plants naturally infected in the field; the symptoms are also very similar to those reported by Whipple (15) for the virus of spotted wilt on *Emilia* in Wisconsin.

3. The virus is easily recovered from immature, diseased tomato fruits, but not from mature, diseased fruits, a feature noted by Samuel *et al.* (12) for the virus of spotted wilt in Australia.

4. All available evidence points to the belief that the virus of yellow spot of pineapple is identical with the virus of spotted wilt, but physical properties have not yet been compared.

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POTATO TUBER NET-NECROSIS AND STEM-END BROWNING STUDIES IN MAINE

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INTRODUCTION

Net necrosis of the potato tuber (*Solanum tuberosum* L.) was described in 1914 by W. A. Orton with the name attributed to Wollenweber (5, p. 14). The fact that net necrosis is an occasional transitory symptom of leaf roll in certain varieties has been demonstrated by Schultz and Folsom (7), A. H. Gilbert (1, 2, and 3), and Quanjer and Elze (6). W. A. Orton described net necrosis as one form of internal stem-end browning, other forms of which were caused by fungi and bacteria. Since then various forms of internal discoloration with nonparasitic causes have been described in a literature that has become extensive and somewhat confused. In Maine and in the seed shipped from Maine, in addition to net necrosis (Fig. 1, A) occasional

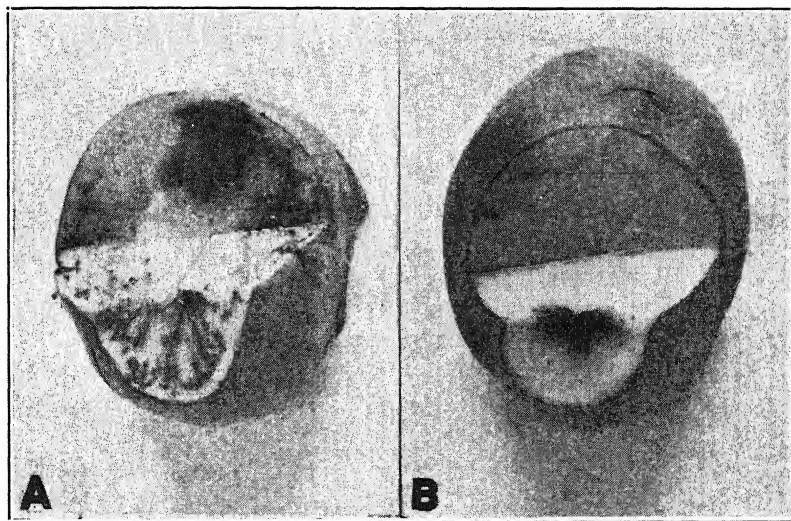


FIG. 1. Green Mountain potato tubers with part of the proximal (stem, butt, or navel) end cut off of each. The deep discoloration in tuber A is net necrosis, an occasional transitory symptom of leafroll in certain varieties, and the comparatively shallow discoloration in tuber B is another form of nonparasitic internal discoloration called "stem-end browning," in Maine.

outbreaks of another type of nonparasitic internal discoloration have given concern, chiefly because it apparently intergrades into net necrosis and because of the common dislike of cooks and seed buyers for any internal discoloration, even if of a type that is completely harmless. This second type of nonparasitic internal discoloration is called "stem-end browning," in Maine and in this paper. Stem-end browning (Fig. 1, B) was observed in

the winters of 1923-24 and 1929-30 and was common enough in some lots of potatoes during the winter of 1937-38 so that studies could be made of it both alone and in comparison with net necrosis. These studies included attempts to relate stem-end browning to different possible causes.

DIFFERENTIATION

Microscopically, stem-end browning usually can be distinguished from net necrosis through the former affecting both phloem and xylem and the latter affecting only the phloem. According to Hill (4) there are other differences.

Macroscopically, the two can often be distinguished with a high degree of success by an experienced person. Fairly successful attempts were made to develop an objective method useful to those with no experience. By recording the depth of penetration of the internal discoloration and its extent at a depth of about 12 mm., or $\frac{1}{2}$ in., in the tubers, and observing the corresponding plants as grown later, it was found that discoloration in more than one ring or zone of the cross section of the tuber at a depth of 12 mm. usually was mild to severe net necrosis (that is, presaged leaf roll), while discoloration extending less than 12 mm. from the stem end of the tuber, or extending to that depth in only the xylem ring, usually was stem-end browning (that is, not associated with leaf roll). The results of one test are given in table 1, and illustrate the degree of accuracy with which the two diseases can be told apart by the half-inch-depth method. If the degree were perfect, the diagnosis of "probably net necrosis" would be accompanied by 100 per cent leaf roll instead of 87 per cent, and the diagnosis of "stem-end browning" would

TABLE 1.—*Accuracy of macroscopic differentiation of stem-end browning and net necrosis*

Diagnosis	Tubers	
	Total no.	Percentage leaf roll
Net necrosis	50	100
Probably net necrosis	143	87
Stem-end browning	286	23
Clear-fleshed	1206	14

be accompanied by 14 per cent leaf roll instead of 23 per cent. The degree is more nearly perfect when, to the rather arbitrary standard just described, there are added other factors on the basis of experience. Stem-end browning is usually darker colored than net necrosis. The discolored strands are fairly continuous in stem-end browning but discontinuous in net necrosis. Finally, net necrosis usually occurs in a greater number of concentric zones than does stem-end browning.

PREVALENCE AND IMPORTANCE OF THE DISEASES

According to the available records, net necrosis was rather scarce in Maine up to 1921 (7). Outbreaks of net necrosis and stem-end browning occurred in central Maine in the crop of 1923, and in central Maine and Aroostook

County in the crop of 1929. A limited survey during the winter of 1929-30 disclosed an average of 5 per cent net necrosis and 9 per cent stem-end browning in thirty stored stocks, with maxima of 16 and 44 per cent, respectively.

In the 1936 crop of potatoes, several stocks in Aroostook County were reported to have considerable stem-end browning. Of these, two had about 10 per cent of the tubers affected and two had about 20 per cent.

In the 1937 crop, an average of about 19 per cent of the tubers were affected with net necrosis in 40 bins of the Green Mountain variety in Aroostook County. Other varieties appeared less affected than Green Mountains. Katahdins and Chippewas have not yet been found with either net necrosis or stem-end browning. This varietal difference in susceptibility to these two types of internal discoloration is not correlated with relative susceptibility to leaf roll or to frost necrosis of the net type. Occasional small outbreaks have occurred in years other than those mentioned.

The infrequency of severe net-necrosis outbreaks in Maine is due to the infrequency of leaf-roll outbreaks in Aroostook County and to the fact that this County is the area of concentrated potato production in the State. In Maine, outbreaks of net necrosis occur more frequently outside of Aroostook County, in correlation with greater spread of leaf roll, and are as severe in percentage of tubers affected, but are less severe in aggregate loss because of the smaller total production of potatoes. Outside of Maine and certain other parts of New England the importance of net necrosis is greatly reduced, in the crop produced there, by the greater use of the less susceptible varieties and by the sale of the crop before the disease has had time to develop. However, wherever seed potatoes are shipped containing net necrosis, the disease is a warning of the presence of leaf roll and, therefore, is important.

RATIO OF NET NECROSIS TO LEAF ROLL

In one study, 29 Green Mountain stocks with 457 tubers apiece on the average were examined for internal discoloration in the tubers and for leaf roll in the vines produced from the tubers. There was a highly significant positive correlation ($r = +0.557$; see 9, table 16, on significant values) between the percentage of net necrosis (averaging 8.8) and the percentage of leaf roll (averaging 23.3) but the ratio between the leaf-roll and net-necrosis percentages in individual lots, averaging about 3.3:1, varied from 1:1 to 9:1. Therefore, while net necrosis in general may indicate how much leaf roll is present in a crop of Green Mountain potatoes, it is not a very reliable indicator of the percentage of leaf roll in all individual lots.

LACK OF EFFECT OF STEM-END BROWNING ON PLANT VIGOR AND YIELD

In the spring of 1937, 3 stored lots of potatoes on as many farms were drawn on for a comparison between plants from stem-end browning tubers and clear-fleshed tubers of the same stock.¹ Two-ounce seed pieces were taken

¹ Unless otherwise stated, the experiments described subsequently in this paper were conducted with potatoes of the Green Mountain variety and on Aroostook Farm, an experimental farm in Aroostook County.

from stem ends and bud ends of both kinds of tubers. Ten seed pieces of each kind were planted at 12-inch intervals in a 10-foot row, replicated in a total of 8 to 10 such plots. The results (Table 2) show that stem-end browning in the seed tuber had no effect on the yield rate of either stem-end or eye-end hills. Under the prevailing conditions, eye-end hills yielded at a significantly higher rate than stem-end hills. This difference between eye-end and stem-end hills was sometimes greater for the clear-fleshed tubers than for the internally discolored ones, which indicated further that stem-end browning is not injurious with respect to yield.

LACK OF PERPETUATION OF STEM-END BROWNING

Many tests over a number of years show neither more nor less stem-end browning in the crop grown from seed with the disease present, than in the crop grown in the same place from other seed free of the disease, with both crops stored in the same place. Also, the crop grown from seed with the disease present in all tubers, selected for the presence of the disease, usually has only a small percentage of affected tubers. Evidently the disease is neither perpetuated in a stock of potatoes, as are some parasitic and virus diseases, nor spread in the field with a consequent outbreak in storage, as occurs with leaf roll causing net necrosis.

On the other hand, stem-end browning appears to be more troublesome on certain farms than on others, and, on those farms, more abundant in certain

TABLE 2.—*Yield rate of hills grown from two-ounce seed pieces from stem ends and eye ends of clear-fleshed tubers and tubers with stem-end browning*

Seed-piece source, etc.	Yield rate in lbs. per hill, ^a etc.		
	Lot 1, Irish Cobbler	Lot 2, Green Mountain	Lot 3, Green Mountain
Stem-end browning tubers, stem end ...	1.47	1.47	1.70
Clear-fleshed tubers, stem end	1.50	1.50	1.67
Difference ^b	0.03	0.03	-0.03
Odds (to 1) ^c	1.00	1.00	1.00
Stem-end browning tubers, eye end	1.65	1.72	1.90
Clear-fleshed tubers, eye end	1.63	1.81	2.03
Difference ^b	-0.02	0.09	0.13
Odds (to 1) ^c	1.00	6.26	7.28
Stem-end browning tubers, stem end ...	1.47	1.47	1.70
Stem-end browning tubers, eye end	1.65	1.72	1.90
Difference in favor of eye end	0.18	0.25	0.20
Odds (to 1) ^c	Over 1350	Over 19,230	37.46
Clear-fleshed tubers, stem end	1.50	1.50	1.67
Clear-fleshed tubers, eye end	1.63	1.81	2.03
Difference in favor of eye end	0.13	0.31	0.36
Odds (to 1) ^c	1350	Very high	Very high

^a At 1 lb. per hill and with spacing of hills 36 by 12 inches, the yield rate per acre would be 242 bushels or 88 barrels.

^b Difference in favor of clear-fleshed tubers unless with —.

^c If the odds are more than 30 to 1, the difference is considered to be significant.

fields than in others. Occasionally, there is a tendency to show progressively more net necrosis from one end of a bin to the other, but there is no such trend with stem-end browning in the same bins. It seems that some environmental condition or set of conditions in the field is the underlying cause of stem-end browning.

TESTING OF POSSIBLE CAUSES OF STEM-END BROWNING

During the outbreak of 1929-30 in Maine, several thousand tubers were examined in several dozen different lots. The stem-end browning was quite general and varied without any apparent relationship to soil, time of digging, presence of any virus disease, region of growth, place of storage, or origin of commercial strain, except that an early-dug lot and a spindle-tuber lot were included in the few lots that were free of the trouble.

In the course of the more recent work, out of the multitude of theories proposed as possible causes of stem-end browning, some of the more plausible were tested. The results will be described briefly.

Soil Type, Source, and Moisture

From 10 different farms where considerable stem-end browning had been found in the potatoes produced the year before, soil was taken that, when analyzed, was found to vary widely in regard to type, fertility, and organic matter content. The soil from each farm was used to fill 5 sections of chimney tile, each about 1 ft. square and 2 ft. long. Two potato plants were grown in each section of tile, fertilized at the rate of $\frac{1}{2}$ ton of 8-16-16 fertilizer per acre. The tile sections were set upright in the ground near each other, on the experimental farm at Aroostook County. In the tubers, after a suitable period of storage, a very small amount of stem-end browning was found in nearly all lots, regardless of the soil in which they had been grown. The soil lots varied from 4.61 to 6.60 in pH, very low to excessive in NO_3 , none to high in NH_4 , very low to high in P_2O_5 , very low to excessive in K_2O and Ca, low to very high in Mg, trace to high in Mn, trace to low in Fe, and very low to high in organic matter.² Apparently, these variations did not cause stem-end browning in the conditions of the test.

On the experimental farm in Aroostook County in the summer of 1937, which was a comparatively hot and dry season for the region, water was applied artificially to 2 rows of potato plants every other day. Samples of tubers from the watered rows and from neighboring dry rows both showed no stem-end browning in storage.

Three potato plants were grown in a loam of high humus content, in the greenhouse in 3 jars with the soil moisture kept nearly constant at 20, 30, and 45 per cent of the weight of the dry soil, respectively. In 2 other similar cultures the water content was caused to fluctuate between 20 and 45 per cent. All tubers produced by these 5 plants were free of stem-end browning.

² Analyzed by D. S. Fink, Associate Agronomist of the Maine Agricultural Experiment Station.

Cumulative Fertilizer Treatment

"Permanent fertilizer plots" on Aroostook Farm had received fertilizer of different ratios from different sources or carriers, with and without lime, broadcast, with and without manure, etc., using different rotations, and had been receiving these same treatments for 10 years. The treatments were as follows:

<i>Rotation</i>	<i>Treatment</i>
Three-year	No fertilizer
Three-year	4- 8- 7—check
Two-year	4- 8- 7—check
One-year	4- 8- 7—check
Three-year	4- 8- 7—chemically pure salts
Two-year	4- 8- 7—broadcast
Two-year	4- 8- 7—plus 20 T. manure
Two-year	4- 8- 7—nitrogen in form of $(\text{NH}_4)_2\text{SO}_4$
Two-year	4- 8- 7—nitrogen in form of NaNO_3
Three-year	4- 8- 7—potash in form of KCl
Three-year	4- 8- 7—as preceding, plus 3000 lbs. ground limestone
Three-year	4- 8- 7—potash in form of K_2SO_4
Three-year	4- 8- 7—as preceding, plus 3000 lbs. ground limestone
Three-year	0- 8- 7
Three-year	2- 8- 7
Three-year	6- 8- 7
Three-year	4- 0- 7
Three-year	4- 4- 7
Three-year	4-12- 7
Three-year	4- 8- 0
Three-year	4- 8- 4
Three-year	4- 8-10
Three-year	4- 8-14

All tubers from these various plots in 1937 were free of stem-end browning.

Samples from many of these plots also had been examined several years previously, at which time no stem-end browning was found in more than two per cent of any sample.

Deficiency in Sulphur, Boron, Iron, and Manganese

In 4 plots the addition of sulphur to the soil at different rates had brought the pH to 4.97, 5.40, 5.60, and 5.95, respectively. Samples from tubers grown here were similar with respect to stem-end browning, which was found to be present in a very low percentage of tubers.

On each of 2 farms that had produced affected potatoes in 1936, borax was applied to the soil in different rows at the rate of 1, 2.5, 5, and 10 pounds per acre, respectively, with each treatment replicated several times. There was no effect on the growth or yield rate of the potatoes, or on the amount of stem-end browning.

A nutritional experiment was started in a greenhouse in February, 1938. Plants were grown in white quartz sand in 2-gallon jars, using nutrient solutions. The nutrient solutions were made from chemically pure salts and were supplied by the constant-drip method (8). Plants were grown that became apparently deficient in boron, iron, manganese, and sulphur, respectively, corresponding to the types of solutions used. Other plants were grown that, apparently, had an excess of aluminum, arsenic, copper,

and chlorine, respectively. In addition to these, an iron-manganese relationship study was carried on. The plants received the following 9 treatments:

<i>Iron</i>	<i>Manganese</i>
Low	Low
Low	Medium
Low	High
Medium	Low
Medium	Medium
Medium	High
High	Low
High	Medium
High	High

All the plants grew fairly well in the sand with the exception of those deficient in boron and those injured by an excess of arsenic or copper; these three sets were considerably stunted. After about 100 days from planting, no more moisture was supplied, so that the plants wilted. The tubers were examined for stem-end browning about a month later, but none of them showed it.

In another experiment several 2-gallon jars were filled with soil from different farms that had produced potatoes with stem-end browning. Others were filled with soil known to be deficient in boron for the production of rutabagas. Chemically pure salts were added to each jar to supply nitrogen, phosphorus, and potash at the rate of 3000 pounds of 4-8-8 fertilizer per acre. One potato plant was grown in each jar. Water was added as needed. No stem-end browning developed in the tubers.

Injury to Plants

The opinion has been expressed that stem-end browning is caused by some form of mechanical injury to the plants; so several methods were used to injure either the tops or the roots. One method employed was late cultivation. After the plants had grown too large to be cultivated without disturbing the roots considerably, some of them were cultivated with as little injury as possible; others were cultivated as close to the plants as possible, breaking off a great many of the roots; and others were left uncultivated as a check. Another simple but harsh method was to jerk each plant upward until some of the roots were broken giving a cracking sound. About half of the foliage was removed from other plants during the latter part of the growing season. Another method of injury tried was to hoe as much dirt away from the plants as possible, leaving the roots and tubers protected from the sun and heat by only a very shallow layer of dirt. Samples were dug and placed in storage from plants killed by late blight. Another block of plants was sprayed late in the season with a sulphuric-acid solution sufficiently strong to kill them. When the tops were thoroughly dead the potatoes were harvested and stored. Other samples were dug and placed in storage from plants that were killed or severely injured by frost. Samples

were dug and stored also from plants that were still green and appeared to be normal in every way.

Except for late cultivation, frost injury, and digging when green, the treatments were all carried out in the same field, and a small amount of stem-end browning was found in each case regardless of treatment, but no more than was found in the untreated check lots. No stem-end browning was found in any of the samples from the field in which the tests were carried out on frost injury and digging when green. Late cultivation had no effect on the development of stem-end browning.

Potted plants were frozen. The tubers when examined several weeks later showed no stem-end browning.

Time and Manner of Digging

Twenty-eight hills of potatoes were dug by hand and each was stored separately. Only 1 or 2 tubers from any hill showed stem-end browning when they were examined in the winter. About half the hills were thus affected. However, the percentage of tubers affected was no higher than where the potatoes were dug by machine and stored in bulk.

EFFECT OF STORAGE DURATION AND TUBER WEIGHT

During the winter of 1937-38, one lot of potatoes, of which about 20 per cent had stem-end browning and about 5 per cent had net necrosis, was divided into several similar parts. The tubers were washed and weighed to the ounce and the tubers of each weight-class were divided equally among the several parts, so that the distribution of tuber weights was identical for the several parts.

A second lot of tubers, with about 9 per cent stem-end browning and about 22 per cent net necrosis, was divided in the same way.

At successive dates during the winter, one of the identical parts of each lot was used to determine whether or not there had been any increase in the amount of stem-end browning or net necrosis. Each disease was recorded as to percentage of tubers affected. In addition, a more objective record of discoloration was made by determining the depth of discoloration both in terms of absolute thickness of the affected part of the stem end and in terms of percentage of total tuber length included in the thickness of the affected part, and by estimating the extent of discoloration in the cross section evident at a depth of about 12 mm. from the stem end. For example, a tuber might have a record of discoloration extending 20 mm. or 16 per cent of the length of the tuber and involving, at the 12-mm. depth, 0.3 of the vascular ring and 0.4 of a radius of the cross section.

By no criterion was there any increase in internal discoloration after the time the study was begun, about December 15. However, the results are only preliminary, because the storage room was not kept at a constant temperature, the successive samples contained only about 250 and 350 tubers each, the study was not started early enough in the season, and seasons may vary.

With each weight-class of tubers divided equally among the several parts and, therefore, treated like every other weight-class during the storage period, the records on all tubers were recombined to determine the relationship between tuber weight and internal discoloration. The results are given in tables 3 and 4 and show that stem-end browning increased in frequency as tuber weight decreased, while net necrosis, where abundant, increased in frequency as tuber weight increased.

TABLE 3.—*Frequency of internal discoloration in weight classes of tubers*

Lot	Item	Weight class and item expressed numerically				
		1 to 3 oz.	4 oz.	5 oz.	6 oz.	7 and more oz.
1	Total tubers	512	562	327	244	388
	Percentage of tubers with stem-end browning	24.8	24.4	18.7	20.1	14.9
	Percentage of tubers with net necrosis	3.6	6.0	3.9	3.7	5.4
2	Total tubers	974	357	237	172	351
	Percentage of tubers with stem-end browning	11.2	9.2	6.3	5.2	4.8
	Percentage of tubers with net necrosis	12.0	21.0	31.2	27.7	39.3

TABLE 4.—*Average tuber weight of tubers with different types of internal discoloration*

Kind of tubers	Average weight of tubers in ounces	
	Lot 1	Lot 2
With stem-end browning	4.61	3.56
All kinds	4.95	4.27
With no discoloration	5.03	3.97
With net necrosis	5.03	5.56

SUMMARY

Net necrosis of the potato tuber, an occasional transitory symptom of leaf roll in certain varieties, is distinguishable in several ways from another kind of nonparasitic internal discoloration called, in Maine, "stem-end browning."

While an outbreak of net necrosis indicates unusual spread of leaf roll in the preceding growing season, the ratio of net necrosis to leaf roll varies greatly with individual seed stocks.

Stem-end browning occurs more in some seasons than in others in any part of Maine, and, when occurring, is usually more abundant in some parts of Maine than in others. It has no effect upon plant vigor and yield rate and is not perpetuated in the tubers from one generation to the next. Through successive seasons it appears more troublesome on certain farms than on others, and in certain fields of those farms than in others.

No correlation was found between stem-end browning and various possible causes such as soil type, previous occurrence in the soil, previous fertilizer treatment of the soil, soil nutrients, pH of the soil, soil water, presence of virus disease, origin of commercial strain, injury to the parent plants, time and manner of digging, and certain storage conditions.

Within a given stored lot, stem-end browning was correlated negatively with tuber weight, and net necrosis was correlated positively. Neither malady increased after December 15 during the winter of 1937-38.

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A TRANSMISSIBLE LEAF-CASTING YELLOWS OF PEACH

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(Accepted for publication December 1, 1939)

The disease here considered first attracted our attention in Green Valley, Solano County, California, in 1932. It has since been seen in several orchards in that valley and in the neighboring Suisun Valley, as well as in 2 orchards in Contra Costa County. Similar symptoms have been seen in 4 other counties. The disease has been seen on Early Crawford, Elberta, Fay Elberta, Muir, and Orange Cling.

There is evidence of appreciable spread in some orchards. In a block of 117 trees, where 37 trees were found affected in August, 1936, symptoms had been seen on a total of 49 trees up to mid July, 1939.

SYMPTOMS

The only tangible early-season symptom of this disease recognized thus far is a slight delay in the time of blossoming of affected trees.

In the Green Valley area, where most of the observations have been made, growth may appear approximately normal, especially in the lower part of the tree, until toward the end of June. The distal parts of severely affected trees or branches usually make little or no growth but fail to present more

specific symptoms during the spring months. Eventually such branches die back, often accompanied by sunburn and invasion by shot-hole borers. A single tree may bear some severely affected branches and others, which are normal in appearance, even after the disease has been present in the tree for several years.

More specific symptoms are seen in leaves from late June through July and August. Irregular portions of the leaf blade turn pale-green to yellow and become brittle, soon separating from the rest of the leaf (Fig. 1). Such areas often are surrounded by a purplish border. Affected leaves gradually take on a greenish yellow cast throughout, become somewhat rolled upward and, beginning with the oldest leaves, are dropped until the entire shoot is bare (Fig. 2). Defoliation may be complete on individual branches by the end of July. Tufts of new leaves may appear later at the tips of shoots in some situations.

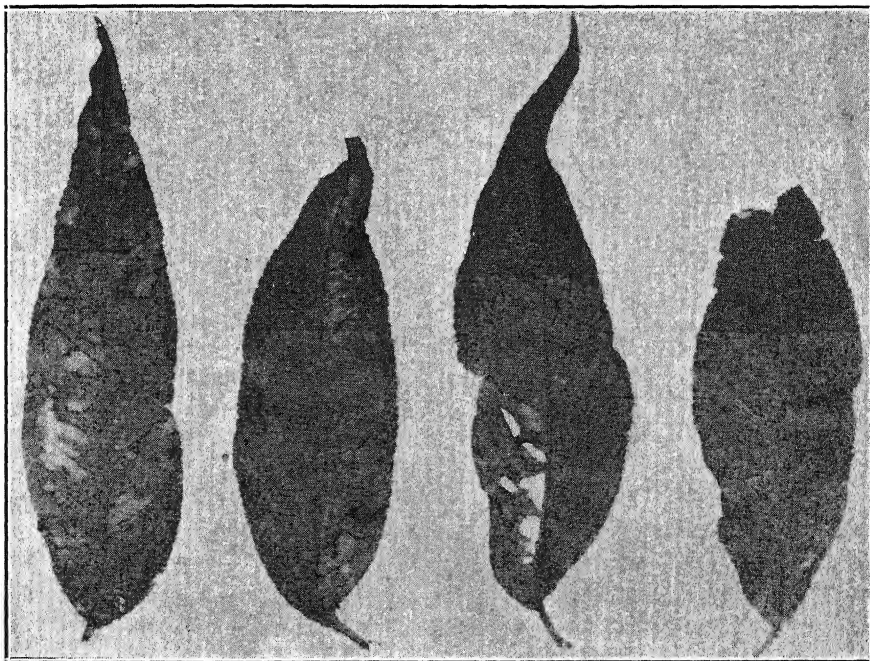


FIG. 1. Leaf symptoms on naturally affected peach, collected in the field, June 20, 1939.

A swelling of the larger veins is commonly present on affected plants in the greenhouse but is rarely seen in the orchards.

More or less coincident with the dropping of leaves, the fruit on affected branches shrivels and drops; on a single tree as harvest approaches there may be on the ground or on the tree all sizes of fruits from small shrivelled mummies to those normal in appearance.

RELATION TO DISEASES OF PEACH IN OTHER AREAS

The characteristics of the disease in California agree closely with those

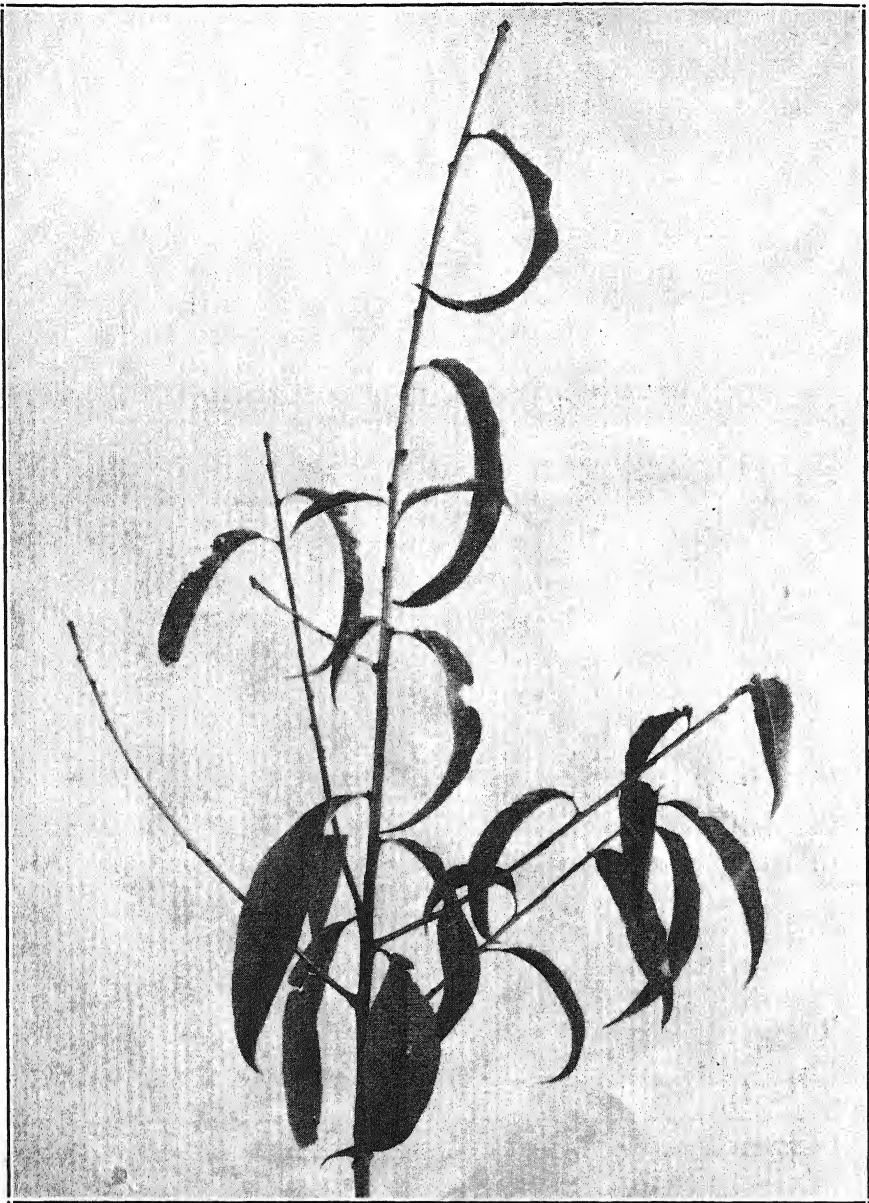


FIG. 2. Characteristic appearance of affected shoots from middle to latter part of summer. Many shoots are entirely defoliated at date of this collection, September 8, 1937.

described by Blodgett¹ in Idaho. He is of the same opinion after having seen some of the affected orchards in California. There is close agreement also with the "X" disease of peach described by Stoddard² in the North-

¹ Blodgett, E. C. Fruit diseases in Idaho, 1936. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 21: 89-95. 1937.

² Stoddard, E. M. The "X" disease of peach. Connecticut (New Haven) Agr. Exp. Stat. Circ. 122. 1938.

eastern States, with two exceptions, namely that all the leaves of affected shoots are dropped in the California orchards including those at the tips and that the percentage of infections when inoculations are made from peach to peach is low with the disease in California. It is probable, however, that the diseases are the same or very closely related.

APPARENT RELATION TO CHERRY BUCKSKIN

The leaf-casting disease of peaches was first noticed in a block of peach trees adjacent to a cherry orchard severely affected by the buckskin disease, the symptoms of which have been described.^{3, 4} More recently cases have been found of association between affected cherries and peaches in 4 separate districts in 2 counties. Partly for this reason, 7 peach trees were inoculated early in 1932 with cherry scions affected by buckskin. When no symptoms appeared in 1932, the same trees were reinoculated in 1933. The inoculated trees continued healthy in appearance in 1934 and 1935, but, in 1936, 4 of them developed symptoms typical of the natural infection on peach, while 3, kept as controls, remained healthy.

This result prompted 2 other experiments, which were made with trees planted early in 1937 and 1938, in each of which 10 Fay Elberta trees were inoculated with buckskin-cherry scions and 10 trees of each lot were left as controls. Here again none of the inoculated trees developed symptoms during the first growing season after inoculation, but, up to October, 1939, 3 trees in one inoculated lot and 5 trees in the other developed clear symptoms of the peach disease, while the 20 control trees remained healthy.

In 1932, 10 cherry trees were inoculated with scions from diseased peach trees. The same 10 trees were again inoculated in 1933 by the same means. None of these inoculated cherry trees showed symptoms of buckskin until 1939 when 2 of the trees showed distinct symptoms of the disease. Since control cherry trees frequently became infected, these results are not considered significant.

In several experiments in the greenhouse a total of 27 apparently healthy cherry trees (*Prunus avium*), including 9 of the Napoleon variety, have been inoculated by inarching or grafting with scions of naturally affected peach. Altogether, 8 of these cherry trees have died, some of them rather suddenly and others after failing to make any growth for several months, but, since the more characteristic symptoms of buckskin are not discernible under the conditions of these tests, the death of the plants is not attributed with certainty to the inoculation. We are, therefore, still uncertain as to the transmissibility of the disease from peach to cherry.

INOCULATION OF OTHER SPECIES OF PRUNUS

Twenty-five plants of the western chokecherry, *Prunus demissa*, transplanted from the wild to pots, were inoculated from peach. Some of the

³ Rawlins, T. E., and W. T. Horne. "Buckskin," a destructive graft-infectious disease of the cherry. *Phytopath.* 21: 331-335. 1931.

⁴ Rawlins, T. E., and K. G. Parker. Influence of root-stock on susceptibility of sweet cherry to buckskin disease. *Phytopath.* 24: 1029-1031. 1934.

grafts were made in November, 1938, and others in February, 1939. The survivors of this experiment grew poorly and all failed to show any clear symptoms up to mid-October, 1939, though one of them bore doubtful symptoms at this time.

During February, 1939, 7 potted chokecherry trees were inoculated with buckskin sweet-cherry scions and 6 were grafted with healthy sweet-cherry scions. Seven were left ungrafted. Of the 7 inoculated trees 2 died and 4 showed a conspicuous carmine (Ridgway) color on the lower leaves when examined on October 2, 1939. This color was more or less continuous along the edges of the leaves and between the main lateral veins. The upper side of the mid-rib and the main lateral veins remained green. None of the 13 control trees showed this symptom distinctly; some did show a tinge of this color in some of the lower leaves. There is some evidence that the reddening of the diseased leaves is preceded by a chlorosis. Since the X disease virus of peach in the Eastern States is reported to cause a reddening of the leaves of infected chokecherries,⁵ our results may perhaps be considered as further evidence that the buckskin disease is identical with or closely related to the "X" disease.

Prunus demissa, regarded by some botanists as a variety of the eastern chokecherry, *P. virginiana*, has not been found thus far in California in the vicinity of affected peach or cherry orchards.

The only native *Prunus* species known to occur in such association are *P. ilicifolia* and *P. subcordata*. The latter has not been inoculated with the peach disease in sufficient numbers even to be suggestive. Both have been inoculated on a small scale with cherry buckskin, but with negative results.

Ten or more plants of each of the following species were inoculated by grafting with affected peach scions, most of them in September, 1938: *Prunus armeniaca*, *P. cerasifera*, *P. communis*, *P. ilicifolia*, *P. mahaleb*. The results to date are negative, but, in view of the low incidence of infection in all the inoculation experiments (see below), cannot be regarded as conclusive.

A few almond and plum trees and a larger number of apricot trees have been seen in close proximity to affected peach trees in orchards, but without any symptoms to suggest that these are affected by the peach disease.

INOCULATION EXPERIMENTS WITH PEACHES

From the beginning, the number of infections resulting from inoculation by grafting peach to peach was low. It was at first assumed that the virus had not had sufficient time to pervade the tree from which the inoculum was taken. When small branches, marked in autumn as bearing symptoms, were found to be uncertain sources of virus the following winter, it became apparent that some other explanation was required. Even the scions used as inoculum often failed to develop symptoms, as well as to infect the inoculated trees.

⁵ See footnote 2.

The results of inoculations and the erratic distribution of symptoms through trees known to have been infected for several years suggested that the virus is not completely systemic. This view is supported by the results of an experiment in which scions were taken from 3 small branches that arose from the same side of a larger branch within a distance of 22 inches. The middle one of these branches bore symptoms in September, 1937, while the other two did not. In February, 1938, 4 scions from the lower branch and 10 from each of the other two were grafted on potted peach seedlings. Up to September, 1939, 3 plants inoculated from the visibly affected branch had developed symptoms, while only 1 plant in the two other groups appeared to be diseased.

Of a few affected shoots or small branches marked in the autumn of 1937 and left on the tree, at least 1 or 2 failed to develop symptoms in 1938. Following this observation, 70 shoots exhibiting clear symptoms were marked in September, 1938, on trees of Early Crawford, Fay Elberta, and Orange Cling. Thirty-three of these were found on July 24 and August 15, 1939, and 11 of these, including some of each variety, appeared to be entirely free of symptoms. This suggests that the virus does not necessarily persist, even in branches that have been invaded by it. Only one case of apparent recovery of an entire tree has been seen, however, and this is still in some doubt.

Several tests were made with roots as a source of the virus. In one of these, 3 pieces of small roots from a diseased orchard tree were grafted on each of 5 nursery trees, which were then planted in a field plot. Five similar trees were inoculated at the same time by grafting affected scions on the tops. After $2\frac{1}{2}$ years 2 of the trees inoculated from shoot scions had shown symptoms, while none of those inoculated by roots had done so, although some of the root pieces used as inoculum were alive for at least 1 year after inoculation.

In another test, pieces of roots from diseased trees were grafted on the tops of 10 seedling peach trees in the greenhouse, and ten similar trees were inoculated by scions from shoots. At the end of 12 months, 4 trees inoculated from roots and 6 inoculated from shoots had developed symptoms. Thus far the roots seem to be less satisfactory than shoots as inoculum.

A few tests have indicated that the Orange Cling variety is a better source of virus than several other varieties. For example, 10 seedling peach trees were inoculated at the same time in the greenhouse with scions of each of the varieties Early Crawford, Fay Elberta, and Orange Cling. During 9 months from the time of inoculation, 3 trees inoculated from Orange Cling displayed symptoms, while none were seen on trees of the other 2 lots.

An experiment was made to test the effect of susceptible and resistant stocks on the expression of symptoms. Successive scions from affected shoots were grafted alternately on potted plants of peach and myrobalan (*P. cerasifera*), i.e., the scion from the base of a shoot was grafted on peach, the next on myrobalan, the third on peach, etc. All told, 50 scions were grafted in

this way. Sixty-five days later, 9 of the scions on myrobalan had developed initial symptoms as against 2 of those on peach roots. Soon afterwards, however, this difference became less striking.

SUMMARY

A graft-transmissible leaf-casting yellows disease of peach, *Prunus persica*, is established in several counties in central California. It is similar to if not identical with diseases of peach in other areas, including the "X" disease of the Northeastern States. There is evidence also that the disease may be caused by the same virus as the buckskin disease of sweet cherry, *Prunus avium*.

Observation of marked branches and grafting experiments indicate that the virus usually is not completely systemic in the tree and is not always present in branches known to have been affected in earlier years. Roots seem to be somewhat less effective than tops as sources of the virus.

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A SOFT ROT OF APPLES CAUSED BY TRICHOSEPTORIA FRUCTIGENA

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(Accepted for publication Nov. 29, 1939)

While collecting diseased apple fruits for class study, H. H. Whetzel of Cornell University, selected among others 2 severely infected McIntosh apples. The fruits had well-developed soft-rot lesions, partially covered with the black, slightly erumpent pycnidia of a fungus (Fig. 1). The color of the skin over the lesions was vinaceous-fawn,¹ glossy and sunken. The infected flesh of the apples was brown and similar in consistency to apples invaded by *Penicillium expansum* Link. A distinct margin separated the diseased and healthy tissues.

As the symptoms conformed to none of those of the common apple fruit rots, the specimens were given to the writer for further study.

THE CAUSAL ORGANISM OBTAINED IN CULTURE

A fungus was obtained in pure culture by planting small pieces of the infected apple tissue on potato-dextrose agar (1.0 per cent) and by pouring dilution plates. The fungi obtained by the 2 methods were identical, and it was later demonstrated that the true causal organism had been isolated. The fungus grew and fruited well on the agar medium, but its pycnidia (Fig. 2, A) were distinctly different in color and shape from those found on the original apple fruits (Fig. 2, B).

¹ Ridgway, Robert. Color standards and color nomenclature. 43 pp., illus. (Washington.) 1912.

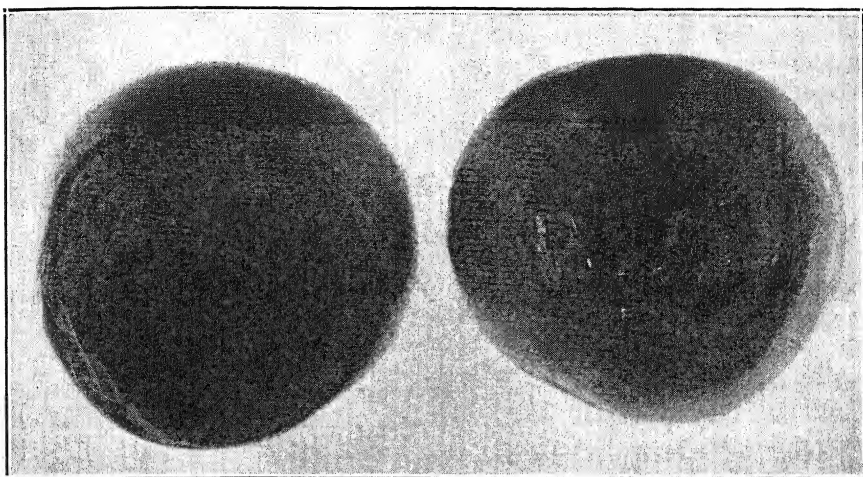


FIG. 1. McIntosh apples on which the pathogen was originally found.

When microtome sections ($18\ \mu$) of the pycnidia were cut and examined, it was found that the erumpent forms taken from the agar culture were covered with a mass of greyish hairs. The pycnidia taken from the apple fruits were nonerumpent or only partly erumpent, black, and without "hairy" covering. It was found during inoculation studies that, under certain conditions, erumpent pycnidia were formed on infected apples. The factors responsible for the development of the two types of fruiting bodies will be discussed later.

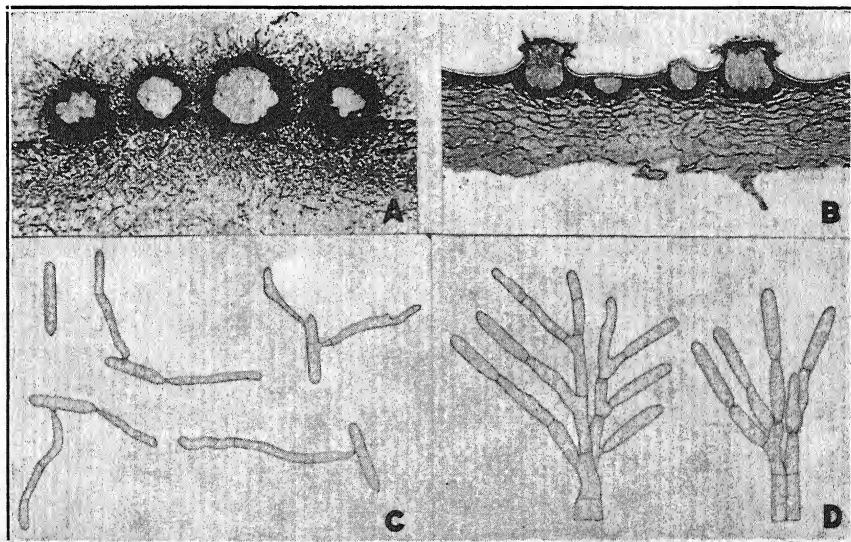


FIG. 2. A. Microtome section of pycnidia produced on potato-dextrose agar. B. Section of pycnidia formed on McIntosh apple. C. Germinated conidia of the pathogen. D. Conidia and conidiophores.

EFFECT OF DEXTROSE ON THE GROWTH AND REPRODUCTION OF THE PATHOGEN

The fungus did not grow or reproduce well on potato-agar without sugar. On test-tube slants, hyphae of about 2 cm. in length developed before growth stopped, and only a few immature pycnidia were formed. When dextrose was added to the medium at concentrations of 1, 2, or 5 per cent, thick, black mycelial mats developed within 10 days at room temperature (about 20° C.). The hyphae in contact with the agar at first were deep green, but later became black. The aerial hyphae were grey.

Drops of a black watery substance formed on many of the pycnidia, particularly on the slants containing 2 and 5 per cent dextrose. In others, conidia oozed from the fruiting bodies and formed in greyish masses at their apices.

When the dextrose content of the medium was increased to 10 per cent the mycelial mats were not so thick as those on media with the lower sugar concentrations. There were also fewer aerial hyphae and the pycnidia, although numerous, were small and immature.

OPTIMUM TEMPERATURE FOR THE GROWTH OF THE PATHOGEN

The optimum temperature for the growth of the fungus was determined from 2 series of tests. In one, the organism was grown on 1 per cent potato-dextrose agar in Petri dishes, the dishes being placed in duplicate in temperatures, varying at 3-degree intervals, from 3° to 30° C.

In another test McIntosh apples were inoculated and placed in duplicate at the same temperatures as the agar cultures. Measurements of the lesions on the fruits and the diameters of the mycelial growths on the plates were made at the end of 8 and 14 days.

The optimum temperature for the growth of the pathogen, as determined from both tests, was found to be near 21° C.

Effect of Temperature on the Production of the Pycnidia

The cultures used in the previous test were removed from the constant-temperature chambers at the end of 21 days and left at room temperature (about 20° C.). In all cases the colonies continued to grow; in time, they completely covered the surface of the agar in the Petri dishes. Later, pycnidia developed abundantly on the mycelium formed at temperatures below 18° C. Only a few pycnidia developed on the mycelium formed at room temperature or on that produced in temperatures above 18° C.

These studies indicate that the optimum temperature for the formation of the pycnidia is lower than it is for vegetative growth.

PATHOGENICITY STUDIES

The pathogenicity of the fungus was determined by inoculating 2 or more fruits from each of 25 varieties of apples. The inoculations were made by cutting a V-shape notch about $\frac{1}{4}$ in. deep in each fruit with a flamed scalpel

and the insertion of a small amount of mycelium taken from an agar culture. After sealing the notch with a gummed paper the apples were placed in glass containers and held at 15° C. for thirty days, when all of the inoculated fruits were found to be severely infected.

The color and size of the pycnidia as well as the abundance of fruiting of the pathogen varied greatly on the apples used in the test. On the variety Arkansas no pycnidia were formed (Fig. 3, A). On Golden Delicious, Jonathan, Northern Spy (Fig. 3, B), and Turley only a few black, partially-erumpent, pycnidia formed at or near the lenticels. With Duchess of Oldenburg (Fig. 3, D), Fameuse, McIntosh, Macoun, Milton, and Wealthy numerous black, partially-erumpent, pycnidia formed on a large part of the apple. On Carleton, Cortland, Gano, Hubbardston, Lobo, Mother, Northwestern Greening (Fig. 3, C), Red Delicious, Red Canada, Rhode Island Greening, Roxbury Russet, Smokehouse, Tolman Sweet, and Twenty Ounce, numerous greyish-erumpent fruiting bodies developed. In all but 2

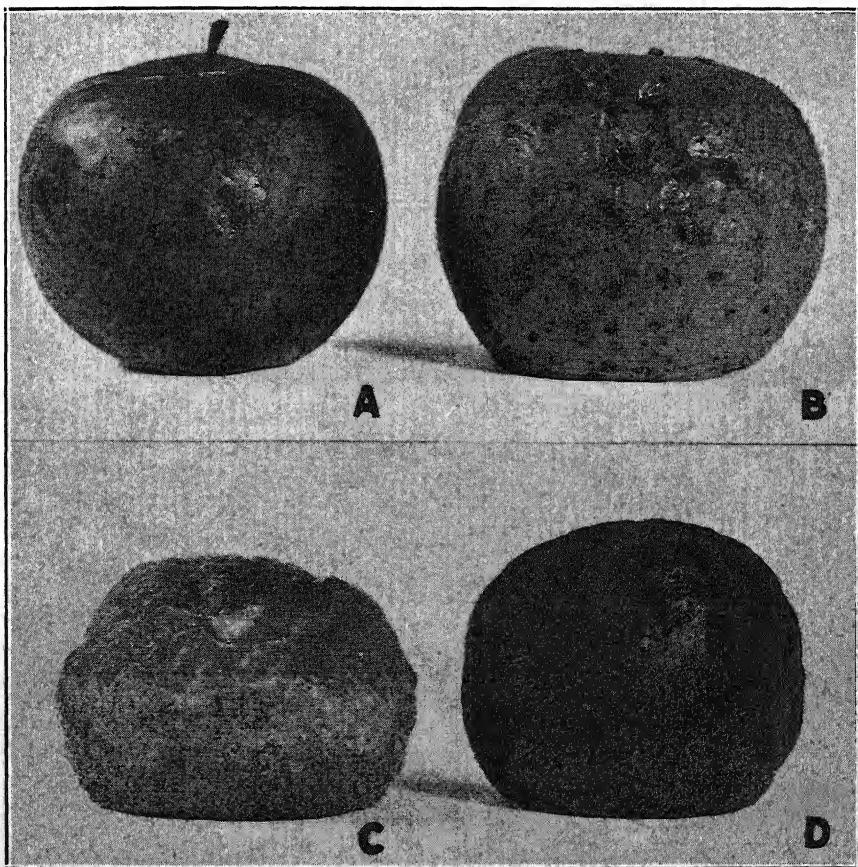


FIG. 3. The formation of pycnidia on four different apple varieties under the same conditions of temperature and relative humidity. A. Arkansas. B. Northern Spy. C. Northwestern Greening. D. Duchess of Oldenburg.

instances the symptoms were the same on both fruits in each of the varieties inoculated.

As the tissue of the fruits became infected the skin on the red varieties changed to a vinaceous-fawn and appeared more waxy than normal. The skins of the yellow and green varieties changed to an ochraceous-buff and a Dresden-brown, respectively. The surfaces of the lesions were dull rather than waxy. At temperatures above 21° C. the skins on the red varieties changed to a dull brown instead of fawn.

Factors Determining the Type of Pycnidia Produced

The number and the kind of pycnidia formed on the inoculated fruits of the 25 apple varieties varied considerably. It was noted, however, that, with the exception of Cortland, the 15 varieties on which the erumpent pycnidia developed were of the late fall or winter sorts all of which have thick skins. Nonerumpent pycnidia developed in all cases on the fruits of the fall varieties that have thin skins.

Later investigations showed that, under certain conditions, those fruits with thin skins lost sufficient moisture during infection and that only nonerumpent pycnidia formed on the surface of the apples.

In several tests where fruits from the thin-skin varieties were inoculated and held in relative humidities (usually above 60 per cent) high enough to prevent excessive loss of moisture from the fruits, erumpent pycnidia formed. At lower humidities the nonerumpent fruiting bodies developed.

Apples with thick skins usually retained sufficient moisture for the production of the erumpent pycnidia. However, when the humidity was low enough to cause sufficient loss of moisture (in one test it was 10 per cent with Baldwin apples), the nonerumpent fruiting bodies developed.

Only as it affects the loss of moisture from infected fruits does temperature within the range of 3–21° C. influence the type of pycnidia formed. In no case, either in culture or on apple fruits, were pycnidia produced by the pathogen in temperatures above 24° C.

Under common apple-storage conditions one may expect to find both the erumpent and nonerumpent types of pycnidia on infected fruits.

The Conidia as Inoculum

The conidia were shown to serve as inoculum when viable conidia of the pathogen were atomized on the uninjured surfaces of McIntosh and Golden Delicious apples, held in moist chambers at 15° C. At the end of 14 days the McIntosh fruits were diseased, and later, fruiting bodies of the pathogen developed on the lesions. The Golden Delicious apples remained unaffected. Where the skins of the fruits from both varieties were punctured before inoculation, with a sterile needle, the fruits of both varieties became infected.

Infected Apple Tissue as Inoculum

Tests showed that when infected apples touched healthy fruits they were sources for spread of the fungus under storage conditions. Brown

lesions developed on McIntosh apples from which the pathogen was isolated, when pieces of infected apple tissue were left on the uninjured skins of the fruits under moist conditions. It is not known whether the fungus will penetrate the uninjured epidermis of the thick skinned varieties.

Twig Inoculations

Attempts to obtain infection on one-year-old and current-season growth of Rhode Island Greening apple twigs, by inserting bits of the mycelium into cuts in the bark, were unsuccessful. The inoculations were made in the greenhouse and the cuts were covered with wax to prevent drying during the test period.

IDENTIFICATION OF THE PATHOGEN

For identification of the pathogen parts of the original apples containing fruiting bodies of the fungus were sent to David H. Linder at Harvard University. There it was identified as *Trichoseptoria fructigena* Maublanc.² He pointed out that this was the first report of the finding of a species of this genus in America. The genus *Trichoseptoria*³ differs from *Septoria* in that the pycnidia formed by species of the first genus have a hairy covering, while those of the latter do not.

Maublanc described the pathogen and the symptom of the disease as follows (translation):

Spots somewhat large, depressed, pale reddish yellow. Pycnidia subepidermal becoming erumpent and then superficial; separate, becoming wavy, confluent, with a twisted, hyaline, hairy, covering. Spores cylindrical, curved, both ends obtuse; granular, with a large central oil drop; rarely 3-guttulate and indistinctly 2 septate, hyaline, 18-23 \times 3-3.5 μ . Conidiophores unequal in length, simple or basally branched. In mature fruits of *Pyrus malus* and *Cydonia vulgaris* in France.

The development of the pycnidia and measurements for the conidia of Maublanc's fungus closely agree with those made on the fungus under observation. The conidia measured were 17.0-23.8 μ long by 2.3-3.4 μ wide. The curved spores, illustrated and described by Maublanc, were observed within the pycnidia only. Conidia liberated from the fruiting bodies were found to be straight, rarely slightly curved and were pointed at the basal end and not obtuse on both ends (Fig. 2, C). Neither germinated nor ungerminated septate conidia were observed by the writer and it was found that the conidiophores (Fig. 2, C) were much more complex than described or illustrated by Maublanc.

With the exception of these few differences the fungus studied by the writer closely resembles *Trichoseptoria fructigena* Maublanc and is considered to be the same.

SUMMARY

A fungus causing a soft rot of McIntosh apples was obtained in pure culture by the writer. It was taken from diseased fruits collected by H. H.

² Maublanc, M. A. *Trichoseptoria fructigena*, nov. sp. Bul. Myc. Soc. France 21: 95-97. 1905.

³ Bender, Harold B. *Trichoseptoria*. The Fungi Imperfecti: Order Sphaeropsidales, p. 7. 1934.

Whetzel. David Linder identified the organism as *Trichoseptoria fructigena* Maublanc.

So far as the writer has been able to determine this is the first report of the finding of this pathogen in North America.

The pathogenicity of the fungus was tested on the fruits of 25 varieties of apples and the signs of the pathogen and the symptoms of the disease were studied and described.

The pathogen grows and reproduces well on 1 per cent potato-dextrose agar. The optimum temperature for growth, both on agar and on apple fruits, is about 21° C. The optimum temperature for pycnidial formation is believed to be somewhat lower.

The effect of temperature and relative humidity on the formation of the pycnidia is discussed.

The conidia of the pathogen and infected apple fruits may serve as inoculum.

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ETHYL MERCURY IODIDE—AN EFFECTIVE FUNGICIDE AND NEMACIDE

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Experience over a period of years with various chemical methods of damping-off control has emphasized the need for some soil disinfectant that will control both pre- and post-emergence damping-off, and remain effective over a period of several months. Particularly is this protection needed for seeds requiring a long germination period and seedlings especially sensitive to soil-borne damping-off fungi. The following representative experiments indicate such surprisingly efficient fungicidal and nemacidal qualities in a product known as DuBay 1155-HH¹ that we are led to believe it meets the above requirements.

Six half flats, 3" × 9" × 18", were filled with a mixture of $\frac{1}{2}$ loam, $\frac{1}{4}$ compost and $\frac{1}{4}$ peat (Fig. 1). Two of these were left nontreated, two were treated with DuBay 1155-HH, at the rate of $1\frac{1}{2}$ grams per square foot,² and the soil of the 2 remaining flats was autoclaved at 30 lbs. pressure for

¹ DuBay 1155-HH was obtained from Bayer-Semesan Company, Inc., and contains as the toxic ingredient 5 per cent ethyl mercury iodide.

² When treating soil, DuBay 1155-HH should first be thoroughly mixed with about 30 to 50 times its weight of dry soil or sand. This concentrated mixture is then spread over the surface, first in one direction and then at right angles, thus securing a uniform covering. A dibble may be used to thoroughly mix this concentrated mixture with the rest of the soil. It is necessary to allow a waiting period of 4 to 7 days, the time varying according to strength of treatment, amount of organic material in the soil and type of seed planted. Great care should be taken to weigh out the proper quantity of DuBay 1155-HH for the medium to be treated. If too much is used root injury is very likely to occur, and may easily prove fatal to the plant. One half gram per square foot or less is sufficient for disinfecting river sand.

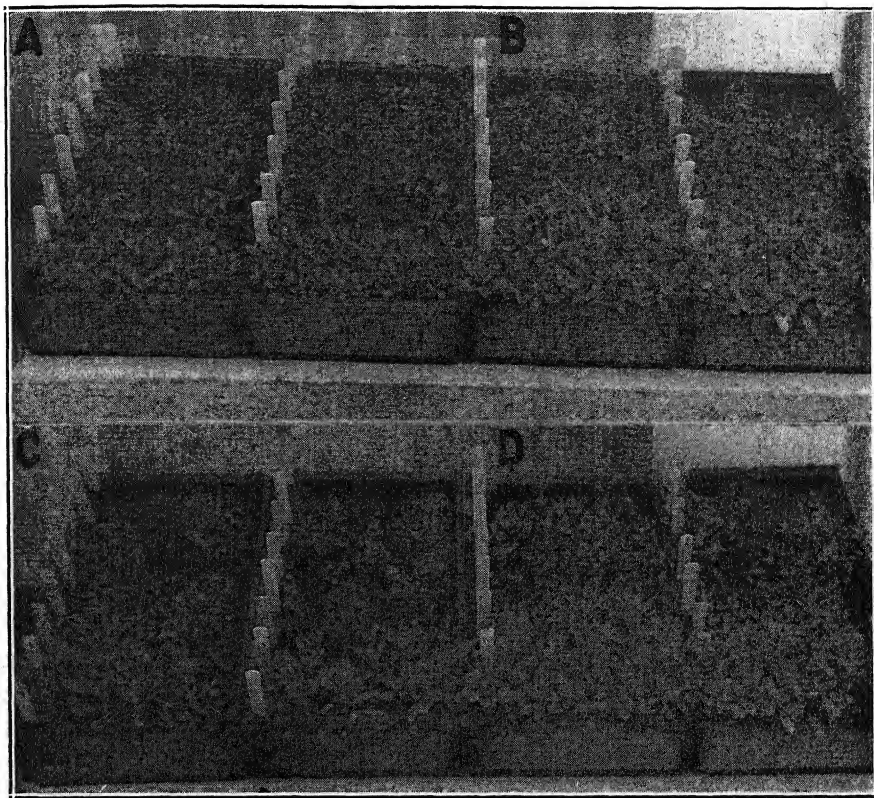


FIG. 1. Effect of soil treatments on seedling emergence and growth. The same soil mixture was used in all flats. A. Soil autoclaved one hour at 30 pounds' pressure. B. Soil treated with DuBay 1155-HH, the same as D. Note larger size of leaves and plants in chemically treated soil. C. Nontreated control. D. Treated with DuBay 1155-HH at the rate of 1.5 g. per sq. ft. Note poor emergence and survival of stocks, phlox, violas and asters in nontreated control. The seeds were planted the same way in all flats.

1 hr. on December 8, 1938. Six days later strips of stocks, *Phlox drummondii*, *Viola cornuta*, White Perfection, and asters, were planted in each flat. Seedlings emerged first in the treated flats, next in the autoclaved soil, and finally, about a week later, in the control. The emergence data are summarized in table 1.

The post-emergence damping-off in the control flats occurred soon after seedling emergence. From 5 to 14 times more seedlings emerged and survived in the treated flats than in the control. Growth was more vigorous than in either autoclaved soil or control. This difference was especially marked about 3 to 4 weeks after emergence, when the photographs shown in figure 1 were taken. In spite of the greater space per plant in the control and in the case of the stocks and phlox in the autoclaved soil as well, the individual plants in the treated soil had more and larger leaves, were much darker green, and bore heavier stems. The stimulation noted after treating soil can hardly be due then solely to suppression of the fungus and resultant

TABLE 1.—*Effect of steam sterilization and soil treatment on seedling emergence as shown by total number of seedlings surviving after different treatments*

Variety seed	Control			Autoclaved 30 lb. 1-hour			Treated 1½ g. of DuBay 1155-HH per sq. ft.		
	Flat 1	Flat 2	Total surv.	Flat 1	Flat 2	Total	Flat 1	Flat 2	Total
Stocks—100 seeds per flat	12-0 ^a	23-0	35	64-0	22-0	86	90-0	60-0	150
Phlox—300 seeds per flat	5-0	6-0	11	12-0	13-0	25	35-0	47-0	82
Viola—200 seeds per flat	40-30	51-38	23	165-0	156-0	321	172-0	152-0	324
Asters—200 seeds per flat	14-10	12-7	9	47-0	27-0	74	43-0	27-0	70

^a Figures to left in each column indicate number of seedlings emerging; those to the right, number that damped off after emergence.

healthier root system; it must be due to some effect of the chemical on either the soil or directly on the plant.

In view of the strong fungicidal properties shown by DuBay 1155-HH in numerous experiments, of which the recent one above reported is typical, the following test of its value as a nemacide was made.

On March 31, 1938, a flat of potting-up soil fairly high in organic content was mixed with five 4-inch pots of soil so heavily infested with nematodes that the *Convolvulus cneorum* plants were almost dead. Roots bearing nematode knots also were included. This soil was then divided into 2 parts and placed in half flats, one of which was treated with DuBay 1155-HH at the rate of 2 g. per sq. ft. and the other was left nontreated. Two hundred seeds of Marglobe tomato were sown in each flat on April 13, 1938. Germination was excellent in both flats. Examination of the plants on May 28, 1938, showed 158 infested in the nontreated control. Most of these plants had the roots completely covered with knots, some bore only a few knots, and 2 plants apparently had escaped infestation. All of the 145 plants in the treated flat were completely free of root knots and were about a third larger than the control plants.

Of each group 24 plants were transplanted into ordinary nontreated soil on May 28, 1938. On July 20, 1938, all the plants from the treated soil were free of root knots, while most of the new roots formed on control plants were infested. Periodic examination of the plants was made and, by October 12th, most of the plants in the control group were dead from the severe infestation. One exceptional plant bore only 8 very small knots. Three of the plants from the treated soil had a few small knots, indicating that they recently had become infested by drainage from the nontreated control plants next to them. The other plants from treated soil were all clean. The striking difference in roots of these 2 groups of plants is shown in figure 2.

The above encouraging results led to the following experiment with cuttings, apparently free of knots, which had been rooted in ordinary non-

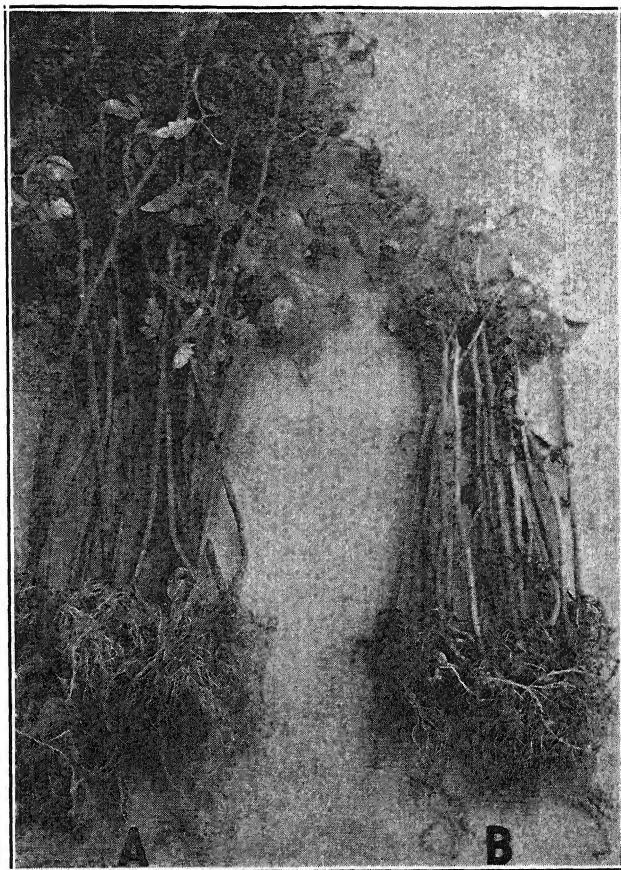


FIG. 2. A. Tomato plants free of root knot after growing in treated soil. B. Those grown in nontreated soil. DuBay 1155-HH was used at the rate of 2 grams per sq. ft. for soil treatment.

treated sand. These were divided into 3 groups and potted up in nontreated, infested soil; infested soil, treated with DuBay 1155-HH at the rate of 2 g. per sq. ft.; and infested soil, treated at the rate of 3 g. per sq. ft. The soils were treated on June 3, 1938, and the cuttings were transplanted to pots on June 18, 1938.

The results as determined by examination on October 13, 1938, are summarized in table 2. No control was run for *Streptosolen* or *Bouvardia* because these species are so susceptible to nematode attack as to show knots, even when the nematode population in the soil is very low. Complete control by treatments of both 2 and 3 g. per sq. ft. was obtained in all, except 3 plants of *Bouvardia humboldti*. The plants in treated soil were transplanted to 4-inch pots on October 15, 1938, containing soil treated at the rate of 2 g. per sq. ft. All were growing very vigorously and the roots were completely free of root knot when examined on December 23, 1938.

TABLE 2.—Summary of results obtained with DuBay 1155-HH applied at indicated strengths to soil heavily infested with nematodes. Plants examined October 15, 1938

Species	Control	2 g. sq. ft.	3 g. sq. ft.
1. <i>Buddleia asiatica</i>	All 12 plants infested. Only about $\frac{1}{2}$ size of those in treated soil	12 plants free of root knots. Very vigorous	12 plants free of root knots. Very vigorous
2. <i>Abelia floribunda</i>	All 13 plants heavily infested	All 12 plants free of root knots	All 9 plants free of root knots
3. <i>Streptosolen jamesonii</i>	No control	All 18 plants free of root knots	All 18 plants free of root knots
4. <i>Ruellia macrantha</i>	All 9 plants heavily infested	All 17 plants free of root knots	All 15 plants free of root knots
5. <i>Bouvardia humboldti</i>	No control	3 plants infested; 2 free of knots	All 5 plants free of knots
6. <i>Bouvardia</i> Coral Gem	No control	All 4 plants free of knots	All 6 plants free of knots

The complete eradication of nematodes from heavily infested potting soil effected by DuBay 1155-HH, in these experiments, has led to large scale commercial treatments of potting soil and tests of its value as a field disinfectant. Experiments testing its value in the control of damping-off in citrus and particularly sensitive species of ornamentals also are in progress. Reports on the results of these tests will be submitted later.

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A MOSAIC DISEASE OF RAPE AND OTHER CULTIVATED CRUCIFERS IN CHINA

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(Accepted for publication Nov. 24, 1939).

A mosaic disease on rape and various other cultivated crucifers is widespread in different districts in China, often causing considerable losses. To determine whether it is identical with any of the virus diseases of crucifers reported from other countries, observations and experiments have been made since 1937 on symptoms, host range, and properties of the virus. The results so far obtained are presented in this paper.

The symptoms of the disease, both in the field and in the greenhouse, are somewhat similar to those of the turnip mosaic on Long Island, New York, as described by Tompkins (4). The infection manifests itself in the beginning as a systemic, conspicuous clearing of veins, usually commencing at or near the base of the leaf and gradually spreading over the entire leaf. Generally, this stage may last for 2 to 3 weeks. In certain cases, particularly under higher temperatures, clearing is soon replaced by vein banding. Dur-

¹ The writers are grateful to Dr. C. M. Tompkins of the California Agricultural Experiment Station, U. S. A., for his kindness in furnishing seed of certain crucifers used in his virus work.

ing the later stages of infection, numerous, raised or non-raised, dark-green islands of irregular outline appear in the chlorotic area between the veins, giving rise to a mottled appearance (Fig. 1, A). All the above-mentioned stages of infection are often accompanied by curvature of the midrib and distortion of the leaf blade. Low temperatures favor the expression of symptoms, and temperatures higher than 20° C. usually induce their masking. Plants infected early are usually severely stunted and often killed, but those infected late in their development are stunted only slightly or not at all.

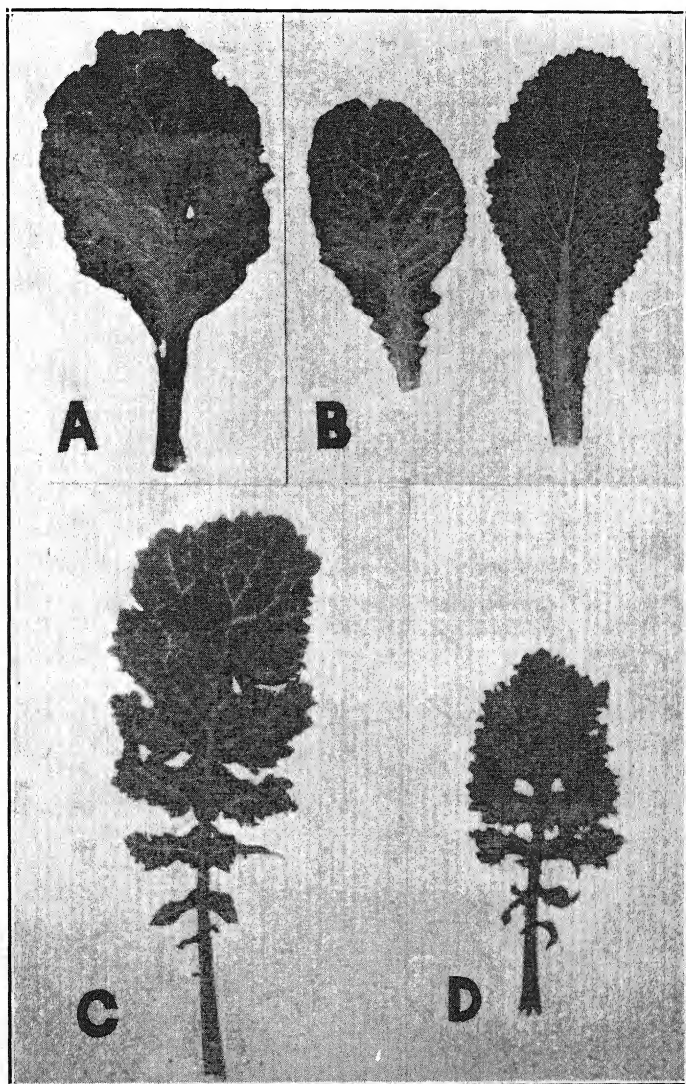


FIG. 1. Symptoms produced by the rape-mosaic virus on leaves of certain crucifers by artificial inoculation in the greenhouse. A. Mottling on rape. B. Vein banding on Chinese cabbage, with noninoculated leaf at right. C. Late stage of vein clearing on turnip. D. Vein banding on turnip.

Attempts to transmit the disease to rape seedlings in the greenhouse by means of rubbing the leaves with expressed juice from diseased plants were unsuccessful. When powdered carborundum (3) was added as an abrasive, infection was obtained in a low percentage of cases. The incubation period ranged from 18 to 26 days. In experiments on insect transmission, only the green peach aphid (*Myzus persicae* Sulzer) was used. Approximately 50 per cent infection was obtained, with the incubation period ranging from 15 to 24 days. Field trials on seed transmission failed to yield positive evidence.

In addition to rape, Chinese cabbage (*Brassica chinensis* L.), turnip (*B. rapa* L.), leaf mustard (*B. juncea* Coss.), and Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey) were found infected with the same virus in nature, the symptoms being identical with those shown by inoculating rape with expressed juices. The results of mechanically inoculating certain cultivated crucifers in the greenhouse are summarized in table 1.

TABLE 1.—*Susceptibility of certain cultivated crucifers to the rape-mosaic virus as determined by artificial inoculation in greenhouse*

Species and common name	Horticultural variety	Number plants inoculated	Number plants infected	Average incubation period, in days
<i>Brassica chinensis</i> L. (pak-choi)	Local (large type)	54	5	29
	Local (small type)	54	0	
<i>B. pe-tsai</i> Bailey (pe-tsai)	Wong Bok	28	3	14
<i>B. oleracea</i> L. var. <i>capitata</i> L. (cabbage)	Local	46	0	22
	Winter Colma	21	0	
<i>B. oleracea</i> L. var. <i>caulorapa</i> DC. (kohlrabi)	Local	48	0	
<i>B. oleracea</i> L. var. <i>botrytis</i> L. (cauliflower)	February	53	0	
<i>B. rapa</i> L. (turnip)	Local	54	11	24
	Purple Top	34	10	
	White Globe	34	10	
<i>B. juncea</i> Coss. (leaf mustard)	Local	52	5	27
<i>Raphanus sativus</i> L. (radish)	White Icicle	31	0	
<i>R. sativus</i> L. var. <i>longipinnatus</i> Bailey (Chinese radish)	Local (round type)	54	24	26
	Local (spring type)	54	10	25

Purple-top White-globe turnip was shown by Tompkins and Thomas (5) to be the best host for separating the viruses of crucifers that they studied. In our study with this particular host, as well as the local variety of turnip, the symptoms produced by the rape-mosaic virus are characterized by systemic, coarse vein clearing (Fig. 1, C), ruffling of the leaf, followed by coarse



FIG. 2. Symptoms produced by the rape-mosaic virus on leaf mustard under natural conditions, showing rugosity and distortion of leaf and stunting of plant. A. Healthy plant. B. Infected plant.

mottling, with raised, dark-green areas interspersed (Fig. 1, D). On Chinese cabbage, a systemic, coarse, and yellowish type of vein banding (Fig. 1, B)

TABLE 2.—*Properties of the rape-mosaic virus*

Longevity in vitro, 11°–13° C.					
Time aged, in hours	Number plants inoculated	Number plants infected	Time aged, in hours	Number plants inoculated	Number plants infected
0	18	11	96	18	2
4	18	6	120	18	3
8	18	3	144	18	0
12	17	2	168	18	0
24	18	4	192	18	0
48	18	6	216	18	0
72	18	2	240	18	0
Tolerance to dilution					
Dilution	Number plants inoculated	Number plants infected	Dilution	Number plants inoculated	Number plants infected
0	36	4	1: 3000	36	2
1: 10	36	5	1: 4000	36	1
1: 100	36	5	1: 6000	36	3
1: 500	36	4	1: 7000	36	0
1: 1000	36	1	1: 8000	36	0
Thermal inactivation point					
Temperature, in degrees C., for 10 min.	Number plants inoculated	Number plants infected	Temperature, in degrees C., for 10 min.	Number plants inoculated	Number plants infected
Control	9	6	65	9	0
50	9	3	70	9	0
55	9	2	75	9	0
60	9	3			

is predominant, often accompanied by the curvature of the midrib and the stunting of the plant. On leaf mustard, the rape-mosaic virus causes pronounced rugosity, distortion of the leaf, and severe stunting of the plant (Fig. 2). Symptoms on Chinese radish are similar to those described for rape. Cabbage and cauliflower, the other two differential hosts used by Tompkins and Thomas (5), failed to show any sign of infection after repeated artificial inoculations.

In the study on the properties of the virus, the local variety of rape was used as the test host. The virus in extracts from rape was inactivated at the end of 6 days after storage at a temperature of 11° to 13° C. The thermal inactivation point for a 10-minute exposure in a water bath was between 60° and 65° C. A tolerance to dilution of 1 to 6,000 was established. The results of different trials are summarized in table 2.

Although similarities in symptoms are found between the rape mosaic and the turnip mosaic (4), the host range serves as a chief differential character. The rape-mosaic virus is unable to infect cabbage and cauliflower. On the other hand, rape is not listed as a host of the turnip mosaic. Further search of the literature reveals only two cases, one from Germany (2) and the other from New Zealand (1), where rape is recorded as being susceptible to virus disease in nature. The descriptions given in both cases, however, are not in agreement with ours either in respect to symptoms or host range. On the basis of the information hitherto available, therefore, our rape mosaic appears to be an undescribed one.

SUMMARY

A description is given of a mosaic disease occurring on rape and 4 other cultivated crucifers in China. Characteristic symptoms consist of vein clearing in the initial stage of infection, followed by vein banding and conspicuous mottling with dark-green areas. Under greenhouse conditions, the virus is transmissible by the green peach aphid and also by mechanical rubbing, with powdered carborundum added as an abrasive. The virus remains infective after a 10-minute exposure to a temperature of 60° C., after storage for 5 days at 11° to 13° C., and after being diluted to 1 to 6,000.

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PHYTOPATHOLOGICAL NOTES

Tomato Fruit Pox.—An abnormal condition affecting green and ripe tomatoes was found in southwestern Texas in 1937, and was mentioned under a tentative name of fruit mottle.¹ The first symptom on green fruits consists of many conspicuous, abnormally dark green dots scattered over the fruit surface. Where numerous, these dots give the fruits a mottled appearance (Fig. 1, A). The dark green spots vary from a very small speck to about 3 mm. in diameter, and are round, elongated, or irregular. Although often distributed at random in the fruit surface, they are more frequently found along the meridians extending from the styler scar to the pedicel (Fig. 1, B). Several of the spots may coalesce and form a streak with its long axis usually oriented meridionally. Later, many of the dark green spots become sunken as pits or pox with ruptured surface tissues (Fig. 1, C, D). This abnormality was named "tomato fruit pox" because of the pock-like marks in the fruit peel.²

The dark green mottle spots are found on fruits of all ages, while most of the pox marks are found on green-wrap, pink, and ripe fruits. The change from the unbroken, dark green spots to the pitted, pox stage occurs in a few days, according to observations of many fruits in the laboratories. Presumably, this change may occur also during transit and storage. As fruits turn pink and red, the dark green spots remain green or turn yellow, while the pox may cork over and appear as an abnormally large lenticel upon the surface of the fruit. Besides making tomatoes unmarketable as first grade fruits, the pox spots may serve as points of infection for fungi and bacteria.

The dark green spots and pox marks appear to be the only symptoms of the trouble, since no other correlated symptoms have yet been found in the plants bearing the affected fruits. Usually all or nearly all of the fruits of affected plants show pox symptoms. More than 90 per cent of the plants were found to be affected in some fields, whereas only a trace of diseased fruits was found in other fields in the same season. Since its discovery, tomato fruit pox has been observed in five seasons of spring and fall crops (1937 to 1939) in the Winter Garden region of Texas. It caused serious economic loss there in the fall of 1938. In some fields, about 10 per cent of the harvested fruit was discarded because of pox injury. Examination of the green-wrap tomatoes at a shipping platform in that season revealed pox symptoms on 10 to 20 per cent of the fruits of some lots. A few affected fruits were found near Jacksonville, Texas, in 1938 and 1939.

The geographic range of tomato fruit pox is partly known. In the winter of 1938-39, the senior writer found pox-affected tomatoes on the

¹ Young, P. A., G. E. Altstatt, and A. L. Harrison. Plant disease survey of southwest Texas. U.S.D.A. Plant Dis. Rptr. 22: 8. 1938.

² Ivanoff, S. S. Tomato fruit "pox." Texas Agr. Exp. Station Fifty-first Annual Report, p. 261, 1938.

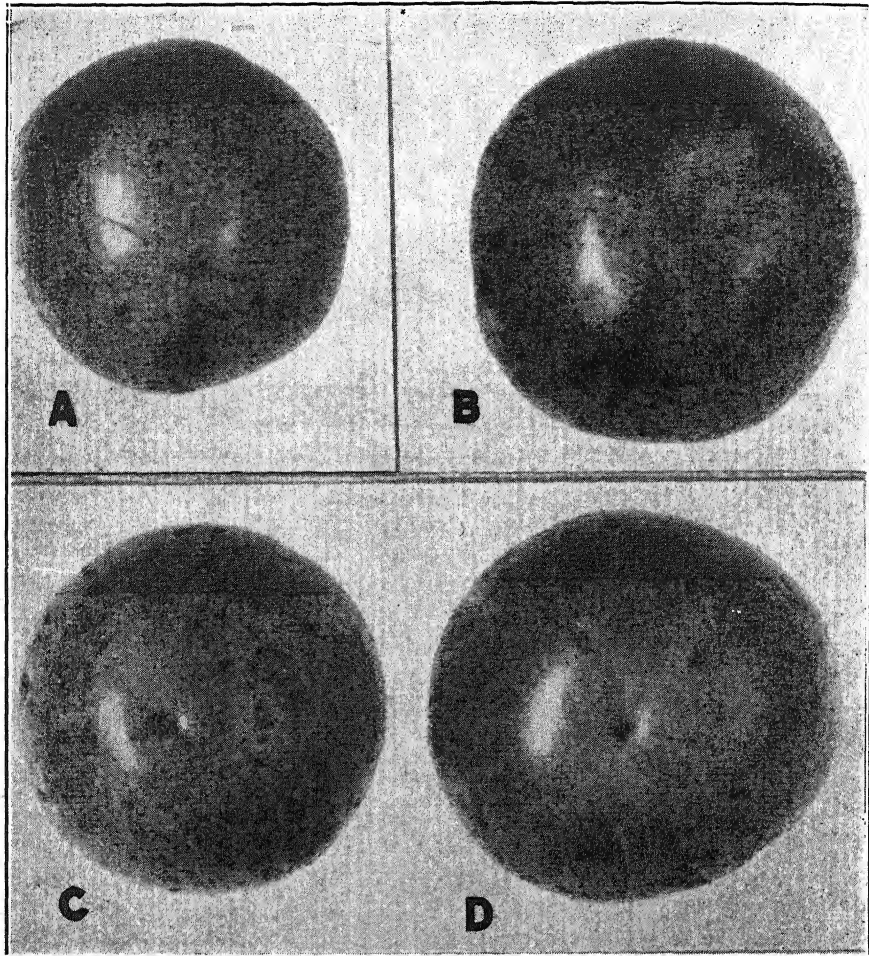


FIG. 1. Tomato fruit pox. A. Green tomato fruit showing numerous small, dark-green areas, the early symptoms of the disease. B. Green fruit showing the same areas grouped mainly along the meridians extending from the styler sear to the pedicel. C and D. Green fruits showing many pits or pox at places where the dark green spots had been. Photographs by L. R. Hawthorn.

markets in Washington, D. C., Virginia, Georgia, and Alabama on fruit shipped in (according to information secured from wholesale dealers) from Florida, Texas, Mexico, Puerto Rico, and Cuba. In the summer of 1939, fruit pox was found to a slight extent affecting Pritchard tomatoes in a few fields in Illinois and Wisconsin.

As to varietal susceptibility, fruit pox in Texas was found most frequently affecting the Pritchard and Stokesdale varieties of tomato, but Marglobe, Rutgers, Bonny Best, Earliana, Summerset, and Globelle also were affected.

The cause of tomato fruit pox is unknown, as far as the writers are aware. No bacteria or fungi have been isolated from affected fruits despite

many attempts to culture a pathogen. Microscopic examinations of affected tissues have not revealed any microorganisms. Apparently, the condition is not hereditary, as it affects many varieties, is found to some extent in many regions, and seed from pox-affected fruits produced plants that bore only normal fruit. It causes serious economic loss of quality in tomato fruit and, therefore, merits further study. The writers would appreciate receiving information about the occurrence of this abnormality in other regions.—S. S. IVANOFF and P. A. YOUNG, Texas Agricultural Experiment Station, Substation No. 19 at Winter Haven and Tomato Disease Laboratory at Jacksonville.

Seedling Stem Blight of Soybean Caused by Glomerella glycines.—Although Lehman and Wolf,¹ in their paper on soybean anthracnose, stated that soybean plants in all stages of development are subject to infection by *Glomerella glycines* (Hori) Lehman and Wolf, their description of the disease is limited to the effect of the parasite on mature plants. In soybean fields in Szechuen Province, West China, it has been observed frequently that seedlings sometimes are killed by the anthracnose fungus soon after emergence. Infection first appears on the cotyledon as darkened cankers and gradually extends downward to the hypocotyl (Fig. 1). The young stem is rotted and after a short time collapses. In case the infection fails to spread as described, the primary leaves, although in contact with the

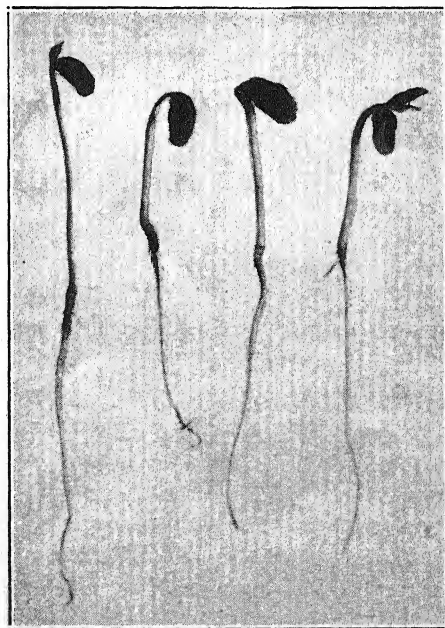


FIG. 1. *Glomerella glycines* on the cotyledons and hypocotyls of soybean seedlings.

¹Lehman, S. G., and Frederick A. Wolf. Soy-bean anthracnose. Jour. Agr. Res. [U.S.] 33: 381-390. 1926.

cotyledons, will not be affected. During wet weather, setose, black acervuli of the fungus are produced abundantly on the lesion.

In a study of parasitism on seedlings, experiments were made with a strain of monosporous origin, designated as 2a. Artificial inoculations in sterilized and nonsterilized soils were made in 3 ways: (1) By soaking the seeds for one hour in a spore suspension of the fungus; (2) by pouring a spore suspension over the soil surface; (3) by mixing the fungus culture with the soil. For each treatment, 100 seeds of the locally used variety of soybean were planted. As shown in table 1, the fungus is able to kill the seedlings in either pre- or post-emerging stages. When seeds were soaked in spore suspension, half or more were killed before emergence and the rest after emergence, so that complete failure of the plants resulted, regardless of whether the soil was sterilized or not. When the inoculum was mixed directly with the soil, the percentage of seedling survival appeared to be higher in nonsterilized than in sterilized soil. Apparently this might be the result of antibiotic action of other soil organisms present. Another series of experiments in which a mixture of 5 strains of the fungus was used gave similar results.

TABLE 1.—*Results of inoculating soybeans with strain 2a of Glomerella glycines*^a

Treatment	Sterilized soil		Non-sterilized soil	
	No. plants emerged	Plants killed after emergence	No. plants emerged	Plants killed after emergence
		No. Per cent		No. Per cent
Seeds soaked in spore suspension	36	36 100	41	41 100
Spore suspension poured over soil	66	62 94	70	27 38
Soil mixed with fungus culture	75	71 95	50	36 72
Check	96	3 3	77	7 9

^a Results are based on quadruplicate pots with a total of 100 seeds in each treatment.

The mycelium of the fungus, either inside the seeds or surviving in the soil, serves as the primary source of infection. Cultures often have been obtained by ordinary isolation methods from seeds 1 to 2 years old. Under experimental conditions, potted soils that were artificially inoculated with cultures from sterilized bean pods in late fall provided active inoculum until the following spring. This has been shown by the appearance of seedlings with typical lesions and the lowering of the percentage of germination of soybean seeds.

Conidia of the fungus are short-lived and very susceptible to drying. The following experiment is representative of several showing similar results. Conidia were washed off the matrix of inoculated bean pods with distilled water, such as might occur during rain under natural conditions. After drying on clean glass slides, germination tests of the conidia were made at intervals of 6 hours. Before drying, 94 per cent of the spores

germinated under standardized conditions; after drying for 6 hours, only 7 per cent germinated. No germination was observed after 12 hours. Even when the conidial masses were permitted to remain in the matrix from which they extruded, after being transferred to and dried on slides, they have failed to germinate after 24 hours. Germination after 6, 12, and 18 hours was 35, 5, and 2 per cent, respectively, in contrast to 96 per cent of germination in the control series.

The ascigerous stage of the fungus has not yet been found in this region.—LEE LING, The Szechuen Provincial Agricultural Improvement Institute, Chengtu, China.

A Leaf Spot of Italian Prune Perpetuated in Budded Stock.—Most Italian-prune orchards observed by the writer in Idaho in the last few years have shown varying amounts of leaf spot and defoliation; reports from other sources indicate that this condition may have been present for many years. The trouble was especially severe in 1936. For the last 3 summers individual trees have been seriously affected, while mild leaf spotting has appeared on most of the trees observed. Attempted isolations and fungicidal treatments have failed to indicate that the spotting is caused by a parasitic fungus.

The symptoms apparently do not appear until early summer. Circumstantial evidence indicates that the spotting comes on rapidly, and the defoliation occurs quickly if the spotting is severe. Of course, reduction in active leaf surface prevents normal photosynthesis and frequently results in

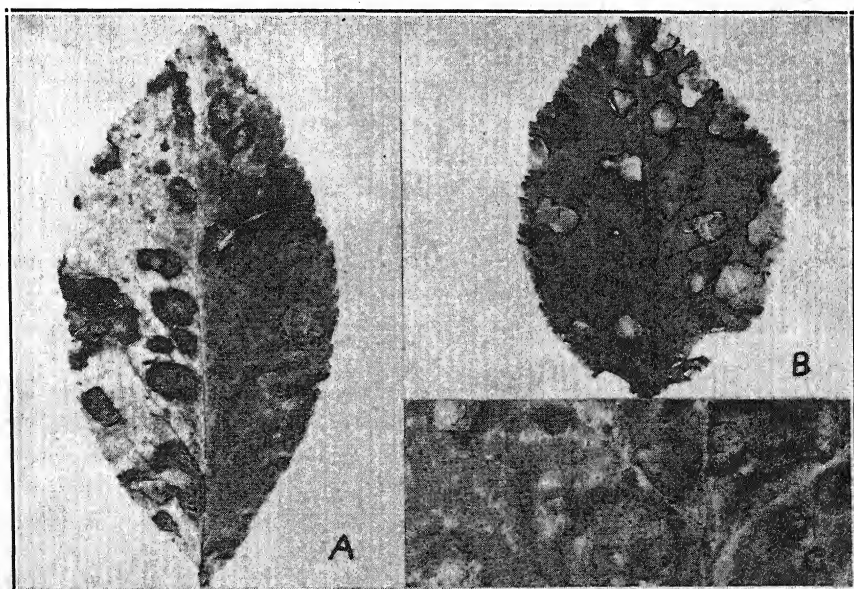


FIG. 1. A leaf spot of Italian prune. A. Leaf from original tree. B. Leaf of shoot resulting from bud from original tree. C. Enlarged portion of leaf similar to A.

heavy fruit drop or a yield of poor fruit. The spots on the leaves vary in size from very small (1–2 mm.) to large blotches and irregular dead areas (Fig. 1). Shot-holing may occur. An indistinct mottling often accompanies severe leaf spot; in some years it may be the only symptom on certain trees. Some observations show that the leaf spot is more severe on trees near the barn lot, chicken runs, or driveways. Affected trees may show very little or extensive terminal growth. It may be of interest to record also that a similar case of serious leaf spot and defoliation has been observed by the writer in the Milton-Freewater district in Oregon.

In August, 1938, bud wood was collected from a tree affected with leaf spot and from a healthy tree in an orchard in northern Idaho and, in September, bud wood from a tree severely affected with leaf spot in southern Idaho. Buds were set on healthy J. H. Hale and Elberta trees at Moscow and observations made during the 1939 season. Shoots of the buds from the healthy prune tree showed no leaf spotting throughout the summer, while severe leaf spot and some chlorosis developed on all shoots from diseased buds. No symptoms appeared on the peach shoots accompanying the diseased prune branches. Unfortunately, no diseased buds were set on prune stock; therefore, there is yet no evidence that the disease factor is transmissible.

The results indicate that the leaf spot and defoliation symptoms, attended by other undesirable factors, in certain Italian-prune trees of Idaho represent either a virosis or a genetic abnormality. The writer believes, however, that environmental factors play an important, although unknown, rôle in the occurrence of this serious trouble.

Further budding work was begun in an attempt to infect healthy prune trees with leaf-spot buds. Additional data may be available next year, but in the meanwhile the trouble is of sufficient interest to warrant this note.—EARLE C. BLODGETT, University of Idaho, Moscow, Idaho.

*A Miniature Root-observation Box.*¹—For the direct observation of root development and root pathology in soil, glass-sided boxes, such as described by Dean,² frequently serve admirably. For detailed work with small plants, where frequent microscopic studies during short periods are required, however, the writer has profitably substituted the much smaller box here described.

This box consists of a U-shape wooden frame with its 3 inner faces grooved to hold 2 wide microscope slide glasses (2 by 3 in.) in parallel planes $\frac{1}{2}$ in. apart. The frame is constructed from strips of sugar pine or similar lumber smoothed to $\frac{7}{8}$ by $\frac{7}{16}$ in., with 2 longitudinal grooves spaced $\frac{1}{2}$ in. apart on one face. These grooves are $\frac{1}{10}$ in. wide and $\frac{1}{8}$ in. deep. From such strips 2 pieces are cut $3\frac{5}{16}$ in. tall to form the sides of the frame. The bottom is a piece $2\frac{1}{8}$ in. long, with a small hole for drain-

¹ Published with the approval of the director as Miscellaneous Paper No. 32 of the Pineapple Experiment Station, University of Hawaii.

² Dean, A. L. Root-observation boxes. *Phytopath.* 19: 407–412. 1929.

age bored through the center. Before assembling, a $\frac{7}{16}$ in. length at the bottom end of each side piece is cut away flush with the bottom of the grooves, forming recesses into which ends of the bottom piece fit. The frame is then assembled with glue and brass screws, with the grooves aligned to permit the glasses to be slid in and out. To permit removal of one glass without sliding, the flange beyond the groove to hold this glass may be cut away from the sides of the frame, but not from the bottom. One end of the glass may then be inserted into the bottom groove, the glass pressed into the recesses in the side pieces, and secured in place with small brads thrust into the wood as glass panes are held in a sash. The finished frames, waterproofed by dipping into a clear lacquer, are light and durable.

Such boxes, with glasses in place, are filled with the desired soil or other medium (black sand³ is particularly favorable for observations of nematodes) and the plant or seed is set close to one glass. They are then held in a slanting position in appropriate boxes or frames that are constructed and disposed to protect roots from light and the soil from overheating while exposing top growth to light. Close attention to watering is naturally required and tops of fast-growing plants may require pruning.

For observation, a box is placed onto the microscope stage and illuminated diagonally from above with a beam of light cooled and focused by a spherical flask of water. For general use a dissecting binocular is advantageous, but, for camera-lucida drawings, photomicrographs, and critical observations, the lower magnifications of a compound microscope are employed.

Such boxes proved distinctly superior to the Petri dishes employed in most of the writer's study of attractiveness of roots for nematodes.³ Their use has also facilitated unpublished studies on the parasitism and development of other nematodes, and promises to be useful in varied studies of root pathology.—M. B. LINFORD, Pineapple Experiment Station, Honolulu, Hawaii.

BOOK REVIEWS

BAWDEN, F. C. *Plant Viruses and Virus Diseases*. 272 p., 37 fig. Price 7 guilders or about \$4. Chronica Botanica Co., Leiden, and G. E. Stechert and Co., New York, 1939.

This publication, prepared by the Virus Physiologist of the Rothamstead Experiment Station, deals primarily with the nature of plant viruses rather than with virus diseases. Considering the enormous advance that has been made in an understanding of the nature of plant viruses during the last six years and the fact that much of the work has been with chemical and physical technics with which most plant pathologists are not too familiar, this critical summary and interpretation should be welcomed. It is probable that there will be disagreement, on the part of those more familiar with the nature of the virus, with the author's conclusions, if one may judge by his conclusions with respect to problems with which the plant pathologist is more familiar, but this is to be expected.

Chapter I is an introductory survey of the virus problem. It includes a tentative definition of "a virus as an obligately parasitic pathogen with at least one dimension of less than 200 μ ," a history of virus diseases, and theories pertaining to them. In a discussion of nomenclature the author points out the obvious impossibility of adopting a

³ Linford, M. B. Attractiveness of roots and excised shoot tissues to certain nematodes. *Helminthol. Soc. Wash. Proc.* 6: 11-18. 1939.

system of nomenclature such as that proposed by James Johnson, which is not based upon a classification of the viruses themselves. Chapters II and III are concerned with "Symptomatology" in which the variation in symptoms that may be produced by a single virus, depending upon the stage of the disease, the strain of the virus, the environment, and the genetics of the plant, are pointed out. The use of symptoms, such as local necrotic spotting, in quantitative studies, is discussed. In Chapter III are discussed X-bodies, crystalline plates, crystals, etc., and the relation of virus to these bodies; and internal changes other than intracellular bodies. In Chapters IV is discussed "Transmission and properties in expressed sap" including grafting, mechanical, insect, and seed transmission, and resistance to aging, etc., and effect of enzymes and chemicals. Chapter V is concerned with the "Relationships between viruses and their insect vectors." Chapter VI takes up "Virus strains, mutation, and acquired immunity." After discussing variations, differentiation of strains, and origin of strains, considerable attention is given to acquired immunity. Without defining what is meant by immunity, the author proceeds to discuss two types. The first, in which a plant in the chronic stage of a disease (a so-called recovered ring-spot tobacco plant) fails to repeat the earlier phases of the disease when reinoculated with the same virus into already invaded tissue; the second, that in which a plant in the chronic stage of the disease (for example, tobacco mosaic) fails to develop a second similar disease when inoculated with a slightly different strain of the same virus into solidly invaded or diseased tissue. The first is evidently based on a misconception of the ring-spot disease as the author considers only ring and line patterns as symptoms, disregarding leaf-edge chlorosis and necrosis, general chlorosis of yellow ring-spot plants, and pollen sterility, (all symptoms which follow so-called recovery) as symptoms of the chronic stage. The second type of "acquired immunity" appears to be identical with the first, except that the term is applied to plants in which the chronic symptoms are more obvious and the virus from which the plant is immune is another strain of the one already present. Obviously, a plant that already has a disease in a virulent form and will continue to have it as it grows cannot be immune from the virus causing the disease; and it is well known that the plant will not be immune from other strains of the same virus if unoccupied tissue can be located in which the second virus can multiply. The phenomenon has uses in the grouping of viruses and is of some possible value in *protecting* plants against more injurious strains of the same virus; but there appear to be no sound grounds for concluding that the plants "have developed an immunity to the disease." It would appear for the present that competition is a sufficient explanation for the phenomenon and that the term protection could well replace immunity.

Chapter VII considers in detail "Serological reactions of plant viruses." The discussion of "Specificity of serological reactions" is of interest in relation to classification of viruses. If antisera are group-specific, then such supposedly unrelated viruses as tobacco-mosaic viruses and cucumber viruses 3 and 4 which, in common with tobacco-mosaic virus, withstand long periods of drying, must be grouped together, a situation that could not be considered under Johnson's proposed system. The discussion of serum absorption experiments would lead one to the conclusion that viruses, not serologically related if tested with their respective antisera, might be proved to be serologically related if tested with a third antiserum. That is if one virus contained antigens a and b and another c and d they would appear to be unrelated, but if a third virus contained antigens b and c it would be related to both groups and consequently all three must be considered related. We might conclude from this that only positive serological reactions can be considered in classification of viruses.

Chapter VIII is a discussion of "Purification of viruses" by chemical means and by high-speed centrifugation. The details of purification methods used with 4 distinct plant viruses are given and the products of purification described. Chapter IX considers the "Properties of purified virus preparations" from the chemical and physical standpoint and Chapter X deals with the "Optical properties of purified virus preparations." The author attempts to keep these discussions on a plane that can be understood by the person not well trained in the technics now being used in the study of viruses. Chapter XI is entitled "The sizes of viruses." Measurement of sedimentation velocities and viscosity and the structure of the virus particles are discussed. In Chapter XII the "Correlation of virus activity with the isolated nucleoproteins" is considered in an attempt to give the evidence for and against the virus being identical with nucleoprotein. Chapter XIII is concerned with the "Physiology of virus diseased plants." Host metabolism and virus movement are considered. In Chapter XIV, "Classification and control," the author discusses the necessity of a knowledge of the virus, its host relations and insect vectors if sound control measures are to be arrived at. Again, a plea is made for a lasting classification and nomenclature of viruses based on the virus itself. General control measures discussed are curative, immune varieties, tolerant varieties, extremely susceptible varieties, protection by noninjurious strains of virus and others. The author is apparently unfamiliar with the progress that has been made in the control of tobacco mosaic by elimi-

nating the use of barn-cured tobacco by workmen, but considers that the first infections are "chiefly from virus present in commercial tobacco or in the soil," sources which have not yet been proved to be of importance.

In the final chapter the origin and multiplication of viruses is discussed. The question as to whether viruses are to be considered living or lifeless molecules cannot, in the author's opinion, be answered until the word life can be accurately defined but this may well come from further studies of viruses. How viruses multiply and how they have arisen are questions that cannot at present be answered.

The plant pathologist who has not been able to follow the voluminous literature on the nature of virus will find this book a very welcome addition to his library, as it gives a clear, concise discussion of most of the literature by one who has been actively engaged in studying the chemistry and physics of the virus.—W. D. VALLEAU.

ASHBY, HELEN, ERIC ASHBY, HAROLD RICHTER and JOHANNES BÄRNES. *German-English Botanical Terminology*. 195 p., 10/—Net in Great Britain—Thomas Murby & Co., London; Max Weg, Leipzig; Nordemann Publishing Co., Inc., New York, \$3.00.

The volume on German-English Botanical Terminology is the third of Murby's German-English terminologies. It presents an introduction to German and English terms used in botany, including plant physiology, ecology, genetics, and plant pathology. A brief survey of botanical science is given in English and German. The manner of presentation consists of short and concise drafts on each subject matter in English on one page and a translation of the same in German on the opposite page. This method makes it very useful for the student. Wherever possible, the German text is primarily a literal translation of the English. In instances where this is not possible, since the same ideas are often expressed differently in German and English, the idiomatic translations are given. A thorough study of the book will acquaint the student with the technical terms used by German- and English-speaking botanists.

Three appendices are included. Appendix I gives the English, Latin, and German names of common, wild and cultivated plants, especially of those growing in Europe. Appendix II presents a list of the most important common names of plant diseases. The name, host, and cause are listed in both English and German. In appendix IIIa are given the abbreviations frequently used in German botanical literature along with the English translation. Appendix IIIb gives the abbreviations frequently used in English botanical literature with the German translation.

The method of presentation of the subject matter is excellent. Since the authors of the work are well-qualified botanists, the volume should find a good reception by students in both English- and German-speaking countries.—OTTO A. REINKING, N. Y. State Agr. Exp. Station, Geneva, N. Y.

REPORT OF THE THIRTY-FIRST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE 1939 COLUMBUS MEETING

The thirty-first annual meeting of The American Phytopathological Society, held in Columbus, Ohio (December 27 to 30, 1939), was one of the most successful and best attended meetings the Society has held. Approximately 325 members were in attendance. Sixty-two new members were elected at Columbus, bringing the active membership roll to 1082, a new high record for membership in the Society.

Nearly 300 attended the Phytopathologists' Dinner at the Neil House, and enjoyed the program arranged by President Orton and his capable coworkers from the Department of Plant Pathology of West Virginia University.

Special conferences were held on plant disease survey, disease resistance in plants, eradicant fungicides, recent studies on fire blight of apples and pears, and laboratory testing of fungicides. The value of cooperation among different branches of plant science was brought out in joint sessions with the American Society of Economic Entomologists, Section G, A. A. A. S., and affiliated Botanical Societies, the Potato Association of America, the Mycological Society, the Association of American Foresters, and the Physiological Section of the Botanical Society of America and the American Society of Plant Physiologists.

The summer meeting will be held in Seattle, Washington, June 17-22, 1940.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1940

Officers:

- CHARLES CHUPP, President (1 yr.), Cornell University, Ithaca, New York.
J. G. LEACH, Vice-President (1 yr.), West Virginia University, Morgantown, West Virginia.
R. S. KIRBY, Secretary (3 yrs. term expires 1940), Pennsylvania State College, State College, Pennsylvania.
H. A. EDSON, Treasurer of the Society and Business Manager of PHYTOPATHOLOGY (3 yrs. term expires 1940), U. S. Department of Agriculture, Washington, D. C.
H. B. HUMPHREY, Editor in Chief of PHYTOPATHOLOGY (3 yrs. term expires 1940), U. S. Department of Agriculture, Washington, D. C.

Councilors:

- H. W. ANDERSON (term expires 1940), University of Illinois, Urbana, Illinois.
J. B. KENDRICK (term expires 1942), University of California, Davis, California.
C. R. ORTON (term expires 1940), West Virginia University, Morgantown, W. Va.
EUBANKS CARSONER (2 yrs. for the Pacific Div.), P. O. Box 31, Riverside, Calif.
GEO. M. ARMSTRONG, (2 yrs. for the Southern Div.), Clemson Agric. College, Clemson, S. C.

Representatives:

- A. A. A. S. Council (1 yr.), L. M. Massey, C. R. Orton.
Elector Group V, Division of Biology and Agriculture, National Research Council (terms expire June 30, 1940), E. C. Stakman (H. P. Barss, alternate).
Tropical Research Foundation (5 yrs. term expires 1940), L. R. Jones.
International Union of Biological Sciences, A. G. Newhall.
Board of Editors, American Journal of Botany (3 yrs. term expires 1940), G. W. Keitt.
Union of American Biological Societies (and Biological Abstracts), H. B. Humphrey and R. S. Kirby (*ex officio*) H. P. Barss, C. W. Bennett, H. A. Edson, G. W. Keitt.

Standing Committees:

- Advisory on Society Activities and Programs.* W. J. Zaumeyer, Chm., F. L. Drayton, A. A. Dunlap, J. A. Pinckard, R. K. Voorhees, J. C. Walker, C. E. Yarwood.
Coordination in Cereal and Vegetable Seed Treatment Research. M. B. Moore, Chm., W. E. Brentzel, H. T. Cook, F. J. Greaney, H. A. Rodenhiser.
Donations and Legacies. E. C. Stakman, Chm., J. G. Brown, N. J. Giddings, N. E. Stevens, R. P. White.
Extension Work and Relations. Luther Shaw, Chm., C. C. Allison, R. J. Haskell, G. W. Keitt, R. H. Porter, O. A. Reinking, R. C. Rose, D. R. Sands, W. B. Tisdale.
Investments. H. A. Edson, Chm., Charles Brooks, Marvin E. Fowler, J. W. Roberts.
Neurology. A. G. Johnson, Chm., M. B. Waite.

- New Memberships and Subscriptions.* R. F. Poole, Chm., J. C. Carter, Kenneth Kadow, R. S. Kirby (*ex officio*), L. D. Leach, R. M. Lindgren.
- Nomenclature and Classification of Plant Viruses.* James Johnson, Chm., C. W. Bennett, Eubanks Carsner, F. O. Holmes, H. H. McKinney, H. H. Thornberry, Freeman Weiss.
- Phytopathological Classics.* H. H. Whetzel, Manager, H. B. Humphrey, Editor.
- Publicity and Public Relations.* C. T. Gregory, Chm., O. C. Boyd, J. H. Jensen, Frank McWhorter, A. G. Newhall, J. A. Pinckard, G. H. Starr, A. J. Ullstrup, G. F. Weber, P. A. Young.
- Standardization of Fungicidal Tests.* S. E. A. McCallan, Chm., R. H. Daines, J. G. Horsfall, K. J. Kadow, J. W. Roberts, C. E. Yarwood, H. C. Young.

TEMPORARY COMMITTEES

- Auditing.* E. E. Clayton, Ross W. Davidson.
- Elections.* Max Gardner, Chairman, C. E. Yarwood, P. A. Ark.
- Resolutions.* G. W. Keitt, Max Gardner, G. H. Coons.

REPORTS OF OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1939

Report of the Secretary. The Society year 1939 opened with 1077 members and closed with 1082, a gain of 5 members. At the Columbus meeting 62 new members were elected. Fourteen former members were restored to the active roll during the year. The Society lost 71 members, 17 by resignation, 5 by death, and 49 by suspension for non-payment of dues. Of the full membership, 151 are paid-up life members and 8 are paying \$10.00 per year toward life membership.

The Society's clearing agency was established to facilitate contact between employable plant pathologists and phytopathological openings from individuals or institutions. Applications were received from 33 plant pathologists desiring positions and from 11 prospective employers. Fifty-seven individual applications were sent to prospective employers. Four plant pathologists reported as having obtained positions through contacts started by the agency.

R. S. KIRBY

Report of the Treasurer. Statement of accounts for the year ending November 30, 1939.

Receipts:

Balance for 1938			\$4296.22
Annual dues:			
1938	\$ 31.90		
1939	2859.08	(\$134.22 life)	
1940	1919.00	(70.00 life)	
1941	1.00		\$4810.98
Voluntary dues			5.00
Interest on savings account			88.08
Items for other accounts included in checks for dues:			
Sales	2.00		
Subscription	6.50		
Classics	1.25		
Dues, Mycological Society	5.00		14.75
To replace checks returned by bank			9.07
Total receipts			4927.88
			\$9224.10

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:

1938	\$1703.30	
1939	1000.00	\$2703.30
Transferred to Sinking Fund (Building and Loan)		104.58
Transferred to PHYTOPATHOLOGY for publication of Society material		369.33
Secretarial work for Secretary and Treasurer		423.75
Expenses of office of Secretary		59.98
Expenses of Membership Committee		12.83
Preprints of abstracts		37.07
Printing		204.40

Stamps and stamped envelopes	48.72	
Contribution to Biologists' Smoker	10.00	
Transferred to PHYTOPATHOLOGY for:		
Sales	2.00	
Subscription	6.50	8.50
Transferred to Lyman Memorial Fund for voluntary dues accrued to date		37.50
Transferred to Mycological Society for dues		5.00
Transferred to Classics		1.25
Collection charges on checks		2.00
Checks returned by bank		10.00
Total expenditures		\$4038.21
Balance on hand		5185.89
		<hr/> \$9224.10

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, is obtained by deducting \$5.00 from each \$10.00 life-membership installment. This fund totaled \$9466.42 at the close of 1938. During the year ending November 30, 1939, it increased to \$9571.00 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection (\$1000.00 at 6%, \$500.00 at 5%)	\$1500.00
Invested with following building and loan associations:	
Arlington & Fairfax Bldg. and Loan, 5%	1000.00
Columbia Permanent Bldg. Ass'n, 4%	510.00
District Bldg. and Loan Ass'n, 4%	1530.00
National Permanent Bldg. Ass'n, 4½%	1000.00
Northwestern Savings and Loan Ass'n, 4%	2000.00
Perpetual Bldg. Ass'n, 4%	1020.00
Prudential Bldg. Ass'n, 4% (interest 17.22)	1088.22
	<hr/> \$9648.22
Less interest due PHYTOPATHOLOGY	77.22
	<hr/> \$9571.00

The Lyman Memorial Fund, obtained from voluntary contributions, totaled \$2809.31 on November 30, 1938. During the period of slightly over a year ending December 9, 1939, this fund increased to \$3003.00, all of which is invested with the Brookland Building and Loan Association at 4%. Of the total amount \$57.56 is interest, available for PHYTOPATHOLOGY.

H. A. EDSON

Report of the Business Manager of Phytopathology. At the close of the year 1938 there were 638 nonmember subscriptions to PHYTOPATHOLOGY, including 5 complimentary. In 1939 there were 39 cancellations and 37 suspensions for nonpayment of dues, a loss of 76. But, with 89 new paid subscriptions, there is a net gain of 13, increasing the list at the close of 1939 to 651. Although PHYTOPATHOLOGY has a mailing list small in comparison with that of other publications, there is a definite effect upon it as a result of present world conditions, as follows: subscriptions to Czechoslovakia have dropped from 4 to 1; to China, from 23 in 1937 to 9 this year; to Japan, from 71 to 61; to Spain, from 11 in 1936 to none this year. We have already received notice of 9 cancellations to be effective for German subscriptions for 1940, more than one third of their total this year. Were it not for Soviet Russia, with a gain of 22, our list would show net decrease. The Soviet now receives 89 copies of our journal each month, compared with 188 for the United States and possessions.

Statement of accounts for the year ending November 27, 1939.

Receipts:

Balance from 1938		\$3510.16
Subscriptions:		
1938	\$ 116.34	
1939	3303.89	
1940	118.10	
1941	11.35	\$3549.68

Member subscriptions:

1938	1703.30	
1939	1000.00	2703.30
Sales of back numbers		479.39
Advertising:		
1938	174.41	
1939	724.68	899.09
Interest on Sinking Fund:		
First-mortgage notes	176.57	
Building and Loan	169.03	345.60
Interest on Lyman Memorial Fund		55.40
Interest on savings account		70.84
Grant from Rockefeller Institute		600.00
From American Phytopathological Society for publication of Society material		369.33
Allowance for reprints by printer		577.16
Payment by authors for excess illustrations		70.99
Reimbursement by printer		1.70
First-mortgage note paid in full		1000.00

Total receipts 10722.48

14232.64

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY:

Vol. XXVIII, No. 12 and Index ...	\$ 867.45	
Vol. XXIX, No. 1	816.90	
No. 2	918.98	
No. 3	569.87	
No. 4	650.23	
No. 5	563.68	
No. 6	713.91	
No. 7	773.09	
No. 8	811.19	
No. 9	587.63	
No. 10	658.83	
No. 11	675.77	\$8607.53
Postage	667.74	
Storage	48.00	\$9323.27
Secretarial work and office expenses, Editor in Chief		372.82
Secretarial work, Business Manager		222.00
Secretarial work and office expenses, Advertising Manager		125.34
Commission, Advertising Manager, 1938		99.58
Stamps and stamped envelopes		67.19
Supplies		12.26
Printing		12.34
Reimbursement Editor in Chief for resetting type of article ...		21.52
Refund subscription and agent's discount		8.45
Reinvestment of principal		1000.00

Total expenditures \$11264.77

Balance on hand 2967.87

14232.64

H. A. EDSON

Report of Auditing Committee for the year ending November 30, 1939. The accounts of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY are in excellent condition. We have checked statements of receipts, expenditures, bank balances, and certificates of investment, and find these correct in every detail.

December 20, 1939

E. E. CLAYTON
ROSS W. DAVIDSON

Report of the Advertising Manager. Contracts for advertisements during 1939 totalled \$990.61, somewhat above the average for the past 8 years. The total number of revenue-producing advertisements was 123, occupying just under 60 pages of the journal and consisting of 21 full-page, 54 half-page, 47 quarter-page insertions, and one of one-eighth page.

There were also 45 nonrevenue-producing advertisements, occupying 38½ pages. These were made up of notices regarding Phytopathological Classics and the Society's clearing agency for employment of plant pathologists, the Directory of Advertisers, and a few complimentary and exchange advertisements.

During 1939, 17 commercial firms and one member of the Society used PHYTOPATHOLOGY as an advertising medium.

The present Advertising Manager wishes to acknowledge the help and cooperation received from Dr. Kirby in turning over the work and in securing the major part of the contracts for the year just past.

AGNES E. MEIER

Report of the Editor in Chief. The 29th volume of PHYTOPATHOLOGY, exclusive of the index, comprises 1077 pages of printed matter, including illustrations, and is classified as follows: One hundred seven articles, 55 notes, 4 reports of meetings, 8 book reviews, 174 abstracts (4 by title only), 247 text figures, 3 plates and 1 frontispiece. From Jan. 1 to Dec. 18, 1939, a total of 194 manuscripts of articles, notes, reports of Society meetings, and book reviews were submitted for publication in our Journal. Three of these manuscripts were withdrawn and three were rejected. Articles now in press number 25: to this should be added 122 abstracts. Additional manuscripts on hand at the time of preparation of this report numbered 47, or a total of 537 typewritten pages.

Your editor and his associates are pleased to report a continued improvement in the quality and general excellence of the manuscripts that have been and are now being submitted for publication in our Journal.

Those who experience delay in realizing publication could save themselves no little annoyance and disappointment if, before submitting their manuscripts, they would devote more and yet more attention to ordinary grammar and to concise, clear presentation of fact and interpretation. Those who contribute papers marred by such sentence construction as the following must not complain of delayed acceptance and publication:

Numerous measurements indicate that except in exposure to sun when garlic leaf temperatures are usually higher than shaded or unshaded air temperatures (though leaves in shade are at a lower temperature than shaded air) and during rain, when leaf temperatures are about equal or slightly higher than air temperatures, garlic leaf temperatures are lower than the temperature of the surrounding air.

Continued care and attention need to be given to the matter of selecting only the best and most necessary illustrations. Nothing whatever is gained by publishing illustrations that do not portray whatever of symptom, structure, or detail they are said in text or legend to show. Those contributing manuscripts containing tables should not leave to the editor the responsibility of composing suitable table headings. It sometimes happens that manuscripts carry tables wholly lacking in headings or any hint in the text as to the intent or purpose of the data presented. Your manuscript is supposed to present the final and finished distillate of your problem. Make sure that it is such before submitting it for publication. Otherwise cultivate the spirit of forbearance.

Acknowledgment is here made to Dr. F. V. Rand for his valued assistance in preparing the index of Volume 29 of our Journal and to the Science Press Printing Co. for its excellent service as printer and publisher of PHYTOPATHOLOGY.

H. B. HUMPHREY

Report of the Manager of Phytopathological Classics for the year 1939. I beg to submit herewith the annual report of my stewardship as Manager of Phytopathological Classics:

Report for the fiscal year beginning December 15, 1938, and ending December 1, 1939.

Classics No. 1: On hand 12-15-38	139	
Sold during year	48	
On hand 12- 1-39		91
Classics No. 2: On hand 12-15-38	343	
Sold during year	50	
On hand 12- 1-39		293
Classics No. 3: On hand 12-15-38	443	
Sold during year	52	
On hand 12- 1-39		391

Classics No. 4: On hand 12-15-38	508	
Sold during year	54	
On hand 12- 1-39		454
Classics No. 5: On hand 12-15-38	773	
Sold during year	79	
On hand 12- 1-39		694
Classics No. 6: Received 2-10-39	1070	
Sold	224	
Given	8	232
On hand 12- 1-39		838
Cash balance on hand 12-15-38	\$276.50	
Receipts during the year	361.28	
Total		\$637.78

Expenditures:

Postage, express, etc.	\$ 18.08
Advertising (postal cards)	16.55
Bank charges75
Classic No. 6: Printing	366.41
Photographs	10.50

Total expenditures 412.29

Balance on hand December 1, 1939 \$225.49

Due on accounts \$ 10.25

H. H. WERTZEL

Report of the Committee on Necrology. During the calendar year 1939 there occurred five deaths of members as follows:

IVAN C. JAGGER, February 16;
L. M. HILL, May 6;
R. E. STONE, June 4;
E. J. PETRY, October 8; and
KAKUGORO NAKATA, November 14.

A. G. JOHNSON
M. B. WAITE

Following the reading of the necrology report, the members present stood for a moment in silence in honor of their departed colleagues.

Report of the Committee on Biological Abstracts and the Union of American Biological Societies. Your Committee is glad to report that during 1939 Biological Abstracts not only demonstrated that it could be maintained successfully, on the new basis put into effect in 1939, without institutional subsidy or endowment, but that it was able to provide an improved and expanded service, thanks to the cooperation of biological societies and of biologists. The plan of publishing Biological Abstracts in sections, as well as in the complete form, was appreciated and taken advantage of by many workers. Section D, *Plant Sciences*, covered plant pathology, plant physiology, ecology (with biometerology), plant anatomy, systematic botany, agronomy, horticulture, forestry, pharmaceutical botany, pharmacognosy, paleobotany, at \$6 per year, including the complete annual indexes.

The price is not to be raised in 1940, but if 30 per cent of the members of any biological society represented in the Union, including our Society, subscribe to Biological Abstracts or to any of its sections, a reduction of \$1 will be made to each subscriber in such society, according to the plan adopted by the Trustees for the year 1940.

Among the gratifying achievements of 1939 is the doubling of the number of research journals covered systematically. In October, 1939, these numbered 1113 and no important biological journal was missed. The contributions of State and Federal research workers in plant and animal science were well covered through Government cooperation. The 18,108 abstracts published made almost an 11 per cent increase over 1938. Effective financial and editorial assistance by the Society of American Bacteriologists resulted in almost 36 per cent expansion in Section C (Microbiology, etc.). Interested groups made

possible better service in the field of biometeorology or bioclimatics, included in the Plant Science Section D. The central staff was increased from 10 to 11 with 5 temporary and 1 volunteer assistant. The number of section editors, able scientists from all biological fields who serve without pay, rose from 141 to 149. The number of copies sent out rose from 1,931 in 1938 to 2,812 in December 1939. Of the latter 1,375 were sections.

Subscriptions during this first year under the new plan brought in almost as much as the total subscription income received the year before under institutional subsidy. A balance on hand from the previous year, and other income, including sale of back volumes, society grants, and individual contributions, were sufficient to meet all 1939 expenses and leave something still in reserve. Rigid economy and efficiency in operation and the devotion of the staff contributed to make this possible. The hope is that the service may be operated permanently on the basis of income from subscriptions. The prices are set as low as possible to give as many as possible a chance to subscribe, being lower than for any similar service in its field. It is hoped that cancellations of foreign subscriptions, attributable to the war will be offset by increased domestic subscriptions.

Abstracts in 1939 appeared with gratifying promptness. For example, 82 per cent of the October abstracts were from 1939 publications. Three years before only 24 per cent in the corresponding number were from the current year. In 1939 the Annual Indexes, of a type considered unsurpassed for reference usefulness, appeared within 7 months, the shortest period on record. All previous indexes were completed and are available.

It is the hope that members of the Society and advanced students in the field will make increasing use of Biological Abstracts. Further developments of the service will depend on income made possible through additional subscriptions.

The other major activity of the Union centered about the newly launched National Association of Biology Teachers started under its auspices. Dr. Oscar Riddle, its sponsor, reported a membership approximating 2,200 at the end of 1939, and a well-established journal, *The American Biology Teacher*, appearing monthly.

HOWARD P. BARSS, Chairman

Report of Committee on Regulatory Work and Foreign Plant Diseases. Recognizing that all regulatory work seeking to prevent the artificial distribution of plant pests, should be based on sound, scientific, factual information, and feeling that such information is not always available, particularly in respect to regulation of foreign commerce, this committee has sought the advice and cooperation of the Division of Foreign Agricultural Service, United States Department of Agriculture.

Your President, C. R. Orton, and your Chairman, interviewed L. A. Wheeler, Chief of the Service, and received a sympathetic audience. We solicited Mr. Wheeler's cooperation in the collection, organization, and dissemination of information on foreign plant diseases. The collection of such information pertaining to causal agencies, methods of dissemination, and economic importance would be accomplished through the regular channels of the consular service; organization through the Division of Foreign Agricultural Service, and the dissemination in the United States to interested individuals through the Bureau of Plant Quarantine and Control, Plant Disease Survey, or other regular agency.

It is realized that it will take time to effectuate the objectives sought. Our present consular service and agricultural attachés are primarily interested in production records of major crops, and other purely economic matters affecting world supplies of major agricultural commodities.

Later conferences indicated that the Division of Foreign Agricultural Service has taken the first step toward educating consular officials to the need and value of information we have requested. The Bureau of Entomology and Plant Quarantine has delivered a series of lectures on the need for such information before a group of new consular appointees. It is hoped that information on phases of foreign plant diseases, essential to effective regulation of foreign commerce for the protection of American agriculture, horticulture, and forestry, will be forthcoming through the consular service in increasing annual increments, as the agricultural attachés become increasingly educated to the need.

Amendments to the Plant Quarantine Act of 1912 were proposed to Congress during the first session of the 76th Congress. A section of these proposed amendments proposed regulation of the promiscuous and unrestricted importation and interstate movement of plant pathogens, as such, in accordance with a resolution adopted by this Association at its St. Louis meeting in December, 1935. This legislation, H.R.4036 and S.1364 is still in committee in both houses of Congress.

Exempted from the provisions of the bill are "field, vegetable, and flower seeds." It is well known that plant pests are carried on seed of this character. The reasons for exemption are apparently (1) lack of information, (2) difficulties involved in port-of-entry inspection for seed-borne pathogens, although in the 1938 list of intercepted plant pests, interceptions of a number of seed-borne fungi are reported. Until more complete infor-

mation is available on world-wide geographic distribution of pathogens capable of seed distribution, and improved techniques are developed for inspecting seed importations, this country will continue to be open to invasion by foreign pathogens brought in on agricultural and horticultural seeds. The importance of this phase of regulatory work can be appreciated when it is realized that the United States imported in 1938 almost 21 thousand tons of grass and forage crop seed, and almost 13 thousand tons of garden and field seed. In the opinion of your committee the regulation of seed imports on a sound biological basis is a vital problem that we cannot afford to disregard. The subcommittee on seed-borne parasites will report progress in this matter.

Various members of the committee and others have informally discussed the possibility of international agreements pertaining to exports and imports of plant material, said agreements basically calling for restriction of export licenses to those exporters qualified for export from a pest-freedom standpoint. The importing country would reserve the right to suspend blanket permission for exports from the licensed exporters of any country if port-of-entry inspection revealed laxity on the part of the country of origin.

An examination of port-of-entry interceptions would indicate almost complete cessation of international trade in plants and plant products capable of propagation, if the importing country availed itself of the above reservation. Reciprocal arrangements between neighboring countries, or between groups of countries of similar interests and geographical situation, as proposed by H. T. Güssow, would be a gradual approach to the above system, and one offering more immediate prospect of attainment.

Because of the geographic distribution of the members of this committee, no meetings have been held. Correspondence among members has been at a minimum. This situation is not conducive to progress. It is respectfully suggested that the committee be revamped with a materially reduced personnel, in an effort to attain a functional committee organization.

It is further recommended that the committee be instructed to investigate the foreign pest records of the major species of plants or seeds imported annually, and report at our next annual meeting.

Respectfully submitted,

RICHARD P. WHITE, Chairman, H. T. GÜSSOW, J. S. BOYCE, W. A. MCCUBBIN,
R. D. RANDS, J. F. ADAMS, E. L. CHAMBERS, AND M. T. MUNN

Report of Extension Work and Relations Committee. The Extension Work and Relations Committee sponsored two activities in 1939: The first was an evening conference on tobacco diseases, held in Greenville, Tennessee, August 9, the occasion being the annual meeting of the Tobacco Disease Council. This meeting was attended by extension plant pathologists from Virginia, Georgia, North Carolina, Ohio, and the United States Department of Agriculture. Most of the discussion was centered around control treatments for tobacco downy mildew.

The second activity was a conference on the subject: "Recent Studies on Fire Blight of Apples and Pears," which was held on the afternoon of December 28, at the Columbus Meeting. The discussion was centered around the epidemiology and control of fire blight. The principal speakers on the program were G. W. Keitt and E. M. Hildebrand. A detailed report of this conference will appear in the Extension Plant Pathologist in the near future. The attendance at this conference was about 75.

LUTHER SHAW, Chairman, CHAS. CHUPP, R. J. HASKELL, A. L. PIERSTORFF,
R. S. KIRBY, E. C. STAKMAN, G. W. KEITT, W. B. TISDALE, I. L. CONNERS

Report of the Committee on Coordination in Cereal and Vegetable Seed Treatment Research, 1939. Experiments were made to determine the effectiveness of certain fungicides in controlling smuts and seedling diseases of wheat, oats, and barley, and to determine the value of some of these fungicides in increasing yields of these crops. These experiments were carried on at 10 stations in the northern United States, and 3 stations in Canada. Seed lots were selected, treated, and packaged at University Farm, St. Paul, Minnesota, and sent to the cooperating stations. At the present time, complete and excellently prepared reports have been received from 6 of these stations.

The experiments were divided into 2 separate groups, i.e., disease-control tests, and yield tests. In the disease-control tests, seed of artificially smutted, susceptible varieties of wheat and oats and of naturally stripe-infected barley was treated with 5 or 7 different fungicides and was planted in 6- or 8-foot rows replicated 3 times. Notes were taken on the development of disease and on seedling stand.

In the yield tests, fewer fungicides were used on commercial varieties of wheat, oats, and barley. The seed was not artificially inoculated but was moderately infected with common seed-inhabiting fungi. Only yield notes were taken on these tests, which were planted in rod rows replicated either 5 or 10 times. Notes on various ecological factors were recorded for both sets of experiments.

Experimental results have not yet been analyzed statistically, but the data indicate that New Improved Ceresan and DuBay 1155-IW are the most effective chemicals for the control of smuts and barley stripe. Leytosan is less effective for the control of barley stripe and is not at all satisfactory for the control of oat smuts. Formaldehyde dusts, which are generally less effective against oats smuts than New Improved Ceresan, under certain conditions, may be more effective. For example, at Wyoming, where 64 per cent of smut developed in the check, there was only 12 per cent smut in the formaldehyde-dust treatments as compared with 23 per cent in the New Improved Ceresan treatment.

Summaries of all of the data will be sent to each of the collaborators as soon as all of the reports have come in and have been analyzed statistically. No conclusions can be drawn from the yield tests without the aid of statistical analyses.

J. G. Horsfall has prepared a summary of "State Recommendations on Vegetable and Flower Seed Treatment," which he has offered to mimeograph and distribute to the various States. From this report it is evident that a wide disparity exists between the recommendations of the different stations.

No experimental work has been undertaken with corn, flax, or any of the vegetables, and no definite plans have been made for the future, but it is recommended that the present experiments be continued. The committee welcomes criticisms on the present work and invites suggestions for experiments with corn, vegetables, and other crops.

M. B. MOORE, Chairman, W. E. BRENTZEL, F. J. GREANEY,
J. G. HORSFALL, H. A. RODENHISER

Report of the Committee on Standardization of Fungicidal Tests. The work of the Committee has been divided into 3 phases, with specially designated subcommittees as follows: (A) Laboratory Methods—S. E. A. McCallan and J. G. Horsfall, in cooperation with F. Wilcoxon and J. W. Heuberger; (B) Field Methods—H. C. Young and K. J. Kadow; and (C) Laws regulating the sale of fungicides—J. W. Roberts, in cooperation with Errett Wallace.

Laboratory and Field Methods. In the furtherance of standardization of laboratory and field methods it is proposed to develop "Standard Methods" as follows: A method having been studied by several different laboratories and found satisfactory will be presented as a "Tentative Method," mimeographed and distributed to members of the Society and others interested. After the method has been adequately tested and reported back to the committee, it will be either (1) adopted as a Standard Method, (2) modified for further testing, or (3) discarded. A Standard Method would be published under the authority of The American Phytopathological Society, Committee on the Standardization of Fungicidal Tests. In the event of studying potential, tentative methods, it becomes necessary to perform original research, such results would become the property of the investigators, to be published as they saw fit. A tentative or Standard Method would not in itself constitute original research, but would cite all previous and original contributions.

The following Tentative Methods have been proposed:

1. Tentative method on a standard Bordeaux mixture for laboratory tests and for determination of Bordeaux coefficient.
2. Tentative method for determination of mean particle diameter of fungicides.
3. Tentative specifications for slide-moist-chamber method of testing protective fungicides.
4. Tentative recommendations on standard spray nomenclature.

Members of the Society and others interested are invited to cooperate by testing and criticizing these tentative methods. Copies may be obtained from the Committee Chairman, Boyce Thompson Institute, Yonkers, New York.

Continued cooperation in developing laboratory and field methods, and in correlation of laboratory and field results are in progress. A cooperative study of methods of evaluating apple foliage injury, embodying original research, was begun and will be continued next season.

A round-table conference on laboratory testing of fungicides was sponsored by the Committee at the Columbus meeting. A large and responsive group attended. Informal reports were given on Standardization of the fungus, factors involved in deposition of sprays, evaluation of results, indications on predicting field performance, and difficulties and inconsistencies of laboratory tests.

Laws Regulating the Sale of Fungicides. The subcommittee, having studied this question, points out the desirability of uniform State and Federal laws governing the sale of fungicides. Manufacturer, wholesaler, retailer, user, and experimenter would be benefited, and regulatory work would be more effective and less expensive. The cooperation of various interested groups to formulate uniform regulations would be desirable. It is further suggested that the service of the plant pathologist to the user of fungicides would be greatly facilitated if on every package of fungicidal material there was (1) a complete statement of composition and (2) a statement of accurate directions for use.

It is recommended that the Society go on record as favoring these suggestions.

It is proposed that the subcommittee continue its investigations by cooperating with various interested groups such as the entomologists, horticulturists, and manufacturers in an attempt to arrive at a mutual basis for formulating uniform regulations.

S. E. A. MCCALLAN, Chairman, J. W. ROBERTS, H. C. YOUNG,
K. J. KADOW, J. G. HORSFALL, C. E. YARWOOD, R. H. DAINES

Report of the Advisory Committee for 1939. The advisory committee on programs and society activities has given attention during the year to numerous suggestions submitted to it by the membership. We have endeavored to sound out society opinion in a number of representative centers on the character of any changes that might be advantageously suggested to the program committee. The most impressive result of this survey is the general satisfaction with our programs and the comparative lack of general criticism.

There is fairly uniform agreement on certain matters, which have been suggested to the council as follows:

(1) The presentation of new material in the form of exhibits might well receive greater emphasis.

(2) The policy of encouraging groups to get together to discuss problems of common interest in special programs or in informal gatherings is appreciated generally. It, of course, goes without saying that this should not expand to the point of jeopardizing the unity of the general program.

(3) While there is not unanimous agreement on the question as to whether or not a policy of enlarging upon the number of invitation papers should be adopted, the majority opinion is decidedly in the negative.

(4) A majority seem to feel that the length of our session is not excessive. There are many of the opinion that too many papers are accepted, although it would appear that this group is still in the minority. The continuance of the democratic spirit that has prevailed in the acceptance of papers from members is very definitely desired. Any increased authority in the hands of a small committee empowered to select or reject papers on their merits apparently would not be approved by the majority. If it becomes necessary in future to restrict the number of papers at a session to a maximum number, it is suggested that this be accomplished by a general rule of limitation, such as the restriction of a number of papers per member.

J. C. WALKER, Chairman, F. L. DRAYTON, A. A. DUNLAP, M. W. GARDNER,
E. L. NIXON, R. K. VOORHEES, W. J. ZAUMEYER

Report of the Membership Committee. This committee has continued its effort to increase the membership in The American Phytopathological Society. A high-pressure type of campaign has been studiously avoided. Whatever progress has been made we owe to the active cooperation of society officers and a large number of individual society members.

The efforts of the committee have been directed along the following several lines:

1. A circular letter has been sent to each member.

2. A representative in each State and Canadian Province has been asked to canvass the local situation and invite suitable former members and nonmembers to join. The local list of members has been sent to each one.

3. Letters have been written to all former members who have been dropped during the last several years because of nonpayment of dues.

4. Letters have been sent to each promising nonmember scientist who has had a paper cited in the current volume of PHYTOPATHOLOGY.

5. Sixty-two new members were elected and 14 former members were reinstated in 1939.

A. J. RIKER, Chairman, J. C. CARTER, KENNETH KADOW,
R. S. KIRBY, R. M. LINDGREN, B. A. RUDOLPH

Report of the Committee on Technical Words. In view of the fact that the International Botanical Congress, scheduled to convene in Stockholm, Sweden, in 1940, has been postponed, your committee on technical words feels justified in submitting two separate and not wholly consistent lists of terms, with the request that these be printed as a part of this report in order that they may be generally available to the members of the Society, and in suggesting that formal action by the Society be deferred until all members have opportunity to appraise the definitions submitted. One list was recently submitted by G. Wilbrink and contains definitions in French, German, and English. The other list is made up of definitions on which the members of this committee are more or less tentatively agreed. In preparing these definitions we have tried to follow the most common and established usage, were it precise enough, otherwise what seemed to be the most correct usage was followed, consideration being given to usage in other countries. We also have tried to make each definition a unit; that is, understandable without references to another definition, unless it referred to one immediately preceding.

To these lists we venture to prefix some observations regarding the value, as well as the intrinsic difficulty, of the interesting task we somewhat rashly undertook. We are encouraged in this procedure by the earlier action of the Committee on Nomenclature of the Ecological Society (see Ecology 20: 331-333. 1939) whose "basic principles" will repay a careful reading. Anyone really interested in principles of definition will find a number of other relevant references in the text of the following paragraphs.

Dr. Wilbrink and the members of this committee will, of course, welcome suggestions, corrections, and additions. Indeed, if criticisms are not forthcoming, the chief purpose of publication will have been missed. It may be well to emphasize in advance, however, that while we do not regard our definitions as final, or indeed, in some cases, as very satisfactory, we believe that even an imperfect definition may be well worth study.

Considering the number and variety of papers published, language should necessarily serve as an aid instead of a hindrance to understanding, that the reader should not have to search for the author's meaning, depend too much on context, or be obliged to "translate" into common usage. Inevitably, the meaning of a word depends in the long run on usage, but definition is only an aspect of usage. A definition is implicit in every use of a word. Explicit definition records and tends to limit usage.

It should perhaps be repeated that we are under no delusion as to the possibility of inducing uniformity of usage. We feel, however, that something may be done to aid in reducing loose use of technical words. Loose usage is not the same as different usage. An author may use words in a sense quite different from the usual, but, if his concepts are clearly explained and his meanings explicitly defined, consistent with each other and consistently applied by him, he cannot be accused of loose usage. Different usage, however, in itself offers special dangers, particularly if it involves coined or little-known words. One of the extreme examples of this in English botanical writing is the subject of comment by L. A. Walford and G. S. Myers in the current number of Copeia, December 26, 1939, page 240.

The whole purpose of language is to convey ideas to other persons. If each person or group used words in a different way, there would be hopeless confusion of meanings. To a lesser degree the same thing happens if a word or a group of words be used in a manner different from the usual. A certain amount of conformity to accepted usage is necessary to understanding. Definitions should be an aid to attaining the necessary minimum of uniformity.

It is obvious that there is a wide difference in the need for exactness in definition and use. The loose use of such terms as *disease* and *epidemic* causes little confusion or misunderstanding, whereas continued misuse of such words as *immunity*, *resistance*, *tolerance* and *kleindusity* (with *resistance* as a catch-all) tends definitely to confuse or mislead readers. The difficulty is, of course, enhanced when the attempt is made to translate such loosely used terms.

As an illustration of the intrinsic difficulty of defining the sort of terms in which we are most interested, take the two definitions of the word *parasite* in the attached lists. One includes and the other excludes the viruses. There is already very good authority for the use of *host* and *parasite* in connection with viruses. Whatever viruses may finally prove to be, they are certainly, as a group, dependent for their existence on the organisms they infect, as absolutely dependent as such obligate parasites as the rust fungi. They are known to multiply or be propagated, and must in some way obtain material for this spread from an organism which would, therefore, function as host for the virus. Yet so to define the word *parasite* as to include them apparently involves either an extension of the term or the assumption that viruses are *living* things.

In an essay on "The meaninglessness of the terms life and living," recently published by the Cambridge University Press in a volume entitled "Perspectives in Biochemistry," N. W. Pirie points out that in scientific nomenclature there is a whole class of ordinary English words whose meaning the scientist rather gratuitously redefines. *Life* and *living* are clearly words that the scientist has borrowed. The loan has worked satisfactorily until comparatively recently, for the scientist seldom cared and certainly never knew just what he meant by these words. Pirie finally arrives at the conclusion that "until a valid definition has been framed it seems prudent to avoid the use of the word 'life' in any discussion about border-line systems."

While one may always temporarily solve a problem by giving it up, and it is possible, though not convenient, to avoid a term such as *life*, there are terms that we need to use, even though we know our concepts may soon change and that any definition probably will soon be modified. Thus, it seems desirable to attempt a definition of *virus* in spite of the general uncertainty as to the nature of viruses.

In P. W. Bridgman's "The Intelligent Individual and Society" (1939) words and their uses are discussed in a number of places. For example, on pages 18, 19, and 24, which lead up to the statement on page 56 that "The difficulty [in the use of words]

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is enhanced by the fact that words in language are part of a stream of activity. The meaning of a word is affected to some extent by the whole context in which it is embedded, and, since this context never exactly recurs, the meaning of the word is never precisely the same." More to the point right here is a specific reference to the question, "What is life?" found on page 78. It follows a discussion of "footless" questions and points out that many questions which on their face have some "objective" significance, in reality are concerned with verbalisms. "For instance, the question 'What is life?' turns out on analysis not to be a question about the external world alone, but a question as to the adequacy of our classification of the objects of the external world into living and dead."

In order to convince oneself that the dilemma at which we have arrived is by no means confined to plant pathology, it is only necessary to read any one of a number of recent scientific books. For example, in the introductory chapter to "The Human Value of Biology," published in 1938 by the Harvard University Press, Johan Hjort quotes and paraphrases Kant to the effect that whereas an arbitrary concept can always be defined, it must be very difficult and perhaps impossible, to define objects in nature. "Consequently the science of mathematics alone possesses definitions."

In spite of all these admitted obstacles, the members of your committee, again urge the publication, as a part of this report, of the lists submitted herewith, and of their continued revision by subsequent committees, lest the Society fall under the condemnation expressed by Lamarek in relation to a quite different subject. (See The Lamarek Manuscripts at Harvard, edited by Wheeler and Barber, page 161). "The majority of men consider only the words they employ without disturbing themselves seriously about the ideas they intend to express. Everyone interprets words to suit himself according to his lights, his tastes and his desires. . . ."

JESSIE I. WOOD

NEIL E. STEVENS

DONALD REDDICK, Chairman

DEFINITIONS SUBMITTED BY THE COMMITTEE ON TECHNICAL WORDS

ATTENUATION: Lessening of the capacity of a parasitic organism or virus to cause disease in the host; reduction in its virulence.

For the opposite process, *restoration* has been used but is not complete in itself, i.e., must be used in the expression *restoration of virulence*. Other words suggested are *reversion*, *revigoration*, *reviviscence*.

CARRIER: An individual invaded by a pathogenic organism or virus without obvious reaction or sign of injury.

DISEASE: Deviation from normal functioning of physiological processes, of sufficient duration or intensity to cause disturbance or cessation of vital activity.

Difficulty or failure in the vital processes of an organism. (Adapted from Link, p. 847.)

HOST: Living organism harboring another organism or virus dependent on it for existence.

HYPERSENSITIVITY: Violent reaction of an organism to attack by a pathogenic organism or virus, with prompt death of invaded tissue preventing further spread of infection.

(Hypersensitiveness.) (Neurogenetic abortion in Wilbrink's list.)

(Intolerance as used for some virus diseases is apparently the same phenomenon.)

IMMUNITY: Freedom from disease, due to lack of qualities permitting or to possession or acquirement of qualities preventing the operation of the pathogenic factor.

With special reference to parasitic diseases: Freedom from attack by a pathogenic organism or virus due to lack of qualities corresponding to its requirements or to possession or acquirement of additional qualities unfavorable to it.

NATURAL IMMUNITY: Immunity due to qualities inherent in an individual.

ACQUIRED IMMUNITY: Immunity acquired during the lifetime of the individual organism (not certainly demonstrated to occur in plants, with the possible exception of certain virus diseases).

IMMUNIZATION: Treatment of an organism designed to render it exempt from attack by a given pathogenic organism or virus; process of acquiring immunity.

IMMUNE: Exempt from disease; not subject to attack by a pathogenic organism or virus. With *from*; also used (erroneously) with *to*; (and, according to Webster's Dictionary, with *against* or *of*). *From* is generally considered to be best usage.

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INFECT: (Of a pathogenic microorganism or virus)—to invade an organism.

(Of an agent)—to affect an organism with a pathogenic microorganism or virus; to bring about infection in an organism.

INFECTED: (Of an organism)—Invaded by a pathogenic microorganism or virus.

Following most precise usage it is preferable to restrict the application of the term *infected* to an organism actually invaded by a pathogenic microorganism or virus. For mere surface contact or mixture with microorganisms, as of spores on seeds, etc., or for inorganic substrata, it seems best to use the term *contaminated*, as contaminated seed, or soil, etc., although many careful writers do use *infected*. The term *infested* is preferred by some to *contaminated* for use in this connection, whereas others following what appears to be the best non-technical English usage confine the application of *infested* to organic or inorganic substrata invaded by larger foreign organisms.

INFECTIBLE: Possessing qualities permitting invasion by a pathogenic microorganism or virus.

INFECTIBILITY: noun.

INFECTION: Process or state of establishment of a pathogenic microorganism or virus in a living organism; state produced in the affected organism by such establishment.

INFECTIOUS: Resulting from invasion by a pathogenic microorganism or virus (of disease); communicable; transmissible.

INFECTIVE: Possessing ability to produce infection; productive of infection.

(Of a microorganism or virus)—possessing ability to invade a living organism.

(Of a vector, medium, etc.)—possessing ability to transmit a pathogenic microorganism or virus.

INFECTIVITY (infectiveness): noun.

INOCULATE: To contaminate or mix, to bring into contact or implant (an organism, culture medium, soil, etc.), with, a microorganism or virus, or material containing either. Used with *with*.

To introduce a microorganism or virus, or material containing either, into (an organism, culture medium, soil, etc.). Used with *into*, sometimes with *to*.

It does not seem worth while to discourage the common and well-established usage with reference to inorganic substrata, often criticized on the grounds that one cannot inoculate that which cannot become diseased.

INOCULATION: noun.

Of (a medium, organic or inorganic) *with* (a microorganism or virus).

Of (a microorganism, etc.) *into* (a medium).

By (an agent or method).

INOCULUM: Material acting or used in natural or artificial contamination with a microorganism or virus.

INOCULA: plural.

KLENDUSITY: Ability of a susceptible variety to escape infection because of possession of some quality preventing or hindering successful inoculation under conditions conducive to infection in other varieties.

PARASITE: Organism or virus existing within or attached to or in intimate association with another living organism, from the functioning tissues of which it derives part or all of the material for its nutrition.

Organism or virus for which the tissues of another living organism serve as substratum and source of nutrition.

PARASITISM: Partial or complete nutritional dependence of one organism or virus on the tissues of another living individual.

The above definitions do not exclude such structures as embryo sacs and the sporophytes of Bryophyta. If this is desired it will be necessary to qualify the phrase *other living organism* by some such phrase as *in whose development or functioning it is not a necessary or normal part*.

PATHOGEN: Parasitic organism or virus whose activity causes disease in the host.

PATHOGENIC: Disease-inciting; possessing ability to induce disease.

PATHOGENICITY: Ability to cause disease.

RESISTANCE: Ability of an organism to withstand or oppose the operation or to lessen or overcome the effects of an injurious or pathogenic factor.

Ability of the host to suppress or retard the activity of a pathogenic organism or virus.

SENSITIVITY: Inability of the affected organism to endure the operation of an injurious or pathogenic factor or the activity of a pathogenic organism or virus without more or less strong reaction, evidenced by varying degrees of symptom expression and damage.

(Sensitiveness.) (Sensibility, in Wilbrink's list.)

SUSCEPT: Organism affected or capable of being affected by a given disease.

SUSCEPTIBILITY: Inability of an organism to oppose the operation or to overcome the effects of an injurious or pathogenic factor.

Inability of the host to defend itself against or to overcome the effects of invasion by a pathogenic organism or virus.

TOLERANCE: Ability of the affected organism to endure the operation of a pathogenic factor or invasion by a pathogenic organism or virus with little or no reaction, as shown by the more or less complete absence of symptom expression and damage.

(In Wilbrink's list: Nonsensibility plus tolerance.)

VIRULENCE: Relative capacity to cause disease; degree or measure of pathogenicity of a parasitic organism or virus.

VIRULENT: Manifesting a high degree of pathogenicity; strongly pathogenic.

AVIRULENT: opposite of virulent.

VIRUS: An obligately parasitic pathogen, capable of reproduction in suitable hosts, *ultra microscopic* and recognizable only because of the visible effects produced in the infected host. (Derived from Bawden's text. See below.)

"... an obligately parasitic pathogen with at least one dimension of less than 200 μ ." (as defined by F. C. Bawden in "Plant Viruses and Virus Diseases" published by the Chronica Botanica Company, Leiden, Holland. 1939.—Further, discussing the possibility of the existence of saprophytic viruses or parasitic viruses that cause no symptoms in any infected organisms, "... until such saprophytes or hypothetical parasites can be shown to resemble the pathogenic viruses in properties more fundamental than size, it would seem preferable to restrict the name virus to agents fulfilling all three requirements of the suggested definition.")

"Probably the majority of scientists at the present time are of the opinion that viruses are high molecular weight proteins capable of reproduction in a specialized medium, that medium perhaps being restricted to living protoplasm" of infected organisms in which they cause disease. (C. W. Bennett, The nomenclature of plant viruses. *Phytopath.* 29: 422-430. 1939.)

The established plural form in English is **VIRUSES**.

According to Holmes (Francis O. Holmes, Proposal for extension of the binomial system of nomenclature to include viruses. *Phytopath.* 29: 431-436. May 1939) the Latin word *virus* was not used in the plural, but if a plural were formed it would be *vira*. He has used this coined Latin plural form as the name of a suggested new organic kingdom, *Vira* (*l.c.*).

VIROSIS: A disease caused by a virus, virus disease.

The use of *virosis*, *viroses*, has been objected to because of suggested possibility of confusion with *viruses*. This possibility seems remote and insufficient grounds for the rejection of a useful and established word.

DEFINITIONS SUBMITTED BY DR. G. WILBRINK

PARASITE: Organism capable of growing and (or) multiplying on or within a living plant, the host, and of deriving part or all of its food from the functioning tissues of this plant, a one-sided nutritive relationship never beneficial and often harmful to the host.

AGGRESSIVITY OR VIRULENCE: Ability to live as a parasite.

PATHOGEN: Parasite, virus or other agent capable of inducing or inciting disease.

PATHOGENICITY: Ability to induce or incite disease.

ATTENUATION: Lessening of pathogenicity.

RESTORATION: Restoration of pathogenicity.

SUSCEPTIBILITY: The sum total of qualities which make a plant a fit host for a given pathogen.

NONsusceptibility: The lack of the above qualities.

RESISTANCE: The sum total of qualities of the host which oppose the development of a given pathogen.

NATURAL PASSIVE OR STATIC RESISTANCE: Resistance which is due to qualities innate to the host prior to the attack and not to reactions incited by the attack.

NATURAL ACTIVE OR DYNAMIC RESISTANCE: Resistance which is due to reactions incited by the attack.

IMMUNIZATION: Treatment applied to a plant in order to increase its resistance or to confer resistance upon it.

INDUCED RESISTANCE: Resistance or increase of resistance if the plant reacts passively in regard to this treatment.

ACQUIRED RESISTANCE: Resistance if the plant reacts actively in regard to this treatment.

SENSITIVITY: Liability of the plant to show more or less violent symptoms of disease.

NONSENSITIVITY: Ability of the plant to endure the development of a parasite or a virus or the influence of another pathogenic agent without showing symptoms of disease.

TOLERANCE: Ability of a plant to endure the development of a parasite or a virus or the influence of another pathogenic agent without showing more than slight symptoms of disease.

ABORTOGENIC NECROSIS: The prompt death of the host tissue at the point of attack of the pathogen, checking the further development of the latter.

NOMENKLATUR PHYTOPATHOLOGIE

PARASIT: Organismus, der die Fähigkeit besitzt in oder auf einer lebenden Pflanze (Nährpflanze oder Wirt) zu leben oder (und) zu multiplizieren und dabei seine Nahrung teilweise oder ganz den funktionierenden Geweben dieser Pflanze zu entnehmen, welche einseitige Nahrungsgemeinschaft nicht vorteilhaft sondern oft schädlich ist für die Nährpflanze.

AGRESSIVITÄT ODER VIRULENZ: Die Fähigkeit um parasitisch zu leben.

PATHOGEN: Parasit, Virus oder sonstige Erreger im Stande Krankheitserscheinungen bei Pflanzen zu erregen.

PATHOGENITÄT: Die Fähigkeit Krankheitserscheinungen bei Pflanzen zu erregen.

ABSCHWÄCHUNG: Verringerung der Pathogenität.

WIEDERHERSTELLUNG: Restauration der Pathogenität.

ANFÄLLIGKEIT ODER EMPFÄNGLICHKEIT: Die Summe der Eigenschaften, die eine Pflanze geeignet machen als Nährpflanze für einen Parasit oder einen Virus zu dienen.

UNANFÄLLIGKEIT ODER UNEMPFÄNGLICHKEIT: Der Mangel an den genannten Eigenschaften.

RESISTENZ ODER WIDERSTANDSFÄHIGKEIT: Die Summe der Eigenschaften der Nährpflanze, welche die Entwicklung oder (und) Vermehrung des Parasiten oder Virus hemmen.

NATÜRLICHE PASSIVE ODER STATISCHE RESISTENZ: Die Resistenz, welche hervor aus Eigenschaften geht, die schon vor dem Angriff des Parasiten oder Virus anwesend waren und nicht aus Reaktionen resultieren, welche der Angriff auslöst.

NATÜRLICHE AKTIVE ODER DYNAMISCHE RESISTENZ: Die Resistenz, welche auf Reaktionen beruht, durch den Angriff erregt.

IMMUNISIERUNG: Behandlung einer Pflanze mit dem Zwecke die Resistenz dieser Pflanze zu erhöhen oder ihr Resistenz zu verleihen.

INDUZIERTER RESISTENZ: Die Resistenz oder Erhöhung der Resistenz, wenn die Pflanze sich bezüglich dieser Behandlung passiv verhält.

ERWORBENE RESISTENZ: Die Resistenz, wenn die Pflanze sich bezüglich dieser Behandlung aktiv verhält.

EMPFINDLICHKEIT: Beschaffenheit der Pflanze um mehr oder wenig starke Krankheiterscheinungen zu zeigen.

UNEMPFINDLICHKEIT: Vermögen der Pflanze die Entwicklung oder (und) Vermehrung eines Parasiten oder Virus oder die Einwirkung eines anderen pathogenen Einflusses zu ertragen ohne Krankheiterscheinungen zu zeigen.

TOLERANZ: Vermögen der Pflanze die Entwicklung oder (und) die Vermehrung eines Parasiten oder Virus oder die Einwirkung eines anderen pathogenen Einflusses zu ertragen und keine oder nur undeutliche Krankheiterscheinungen zu zeigen.

ABORTOGENE NEKROSE: Das schnelle Absterben des Gewebes der Nährpflanze an der Angriffsstelle des Parasiten oder Virus, wodurch der Entwicklung des Parasiten oder Virus ein baldiges Ende gesetzt wird.

NOMENCLATURE PHYTOPATHOLOGIE

PARASITE: Organisme étant capable de se développer ou (et) de se multiplier sur ou dans une plante vivante (plante nourricière ou sujet) et d'extraire une part de sa nourriture ou toute sa nourriture des tissus fonctionnantes de cette plante, cette association nutritive uni-latérale n'étant pas avantageuse mais souvent nuisible au sujet.

AGRESSIVITÉ OU VIRULENCE: La capacité de se développer ou (et) de se multiplier dans une plante vivante.

PATHOGENE: Parasite, virus ou autre cause capable d'engendrer ou d'inciter des symptômes de maladie végétale.

PATHOGENICITÉ: Capacité pathogène.

ATTÉNUATION: Affaiblissement de la pathogénicité.

RESTAURATION: Restauration de la pathogénicité.

RÉCEPTIVITÉ: Le total des qualités, qui rendent une plante accessible à un parasite ou un virus.

RÉSISTANCE: Le total des qualités du sujet, qui s'opposent au développement ou (et) à la multiplication du parasite ou virus.

RÉSISTANCE NATURELLE PASSIVE OU RÉSISTANCE STATIQUE est basée sur des qualités déjà présentes dans le sujet antérieurement à l'attaque du parasite ou du virus et non produites par l'attaque.

RÉSISTANCE NATURELLE ACTIVE OU RÉSISTANCE DYNAMIQUE est basée sur des réactions produites par l'attaque.

IMMUNISATION: Traitement d'une plante afin d'en augmenter la résistance ou de lui conférer de la résistance.

INDUITE est cette augmentation ou obtention de résistance d'une plante passive en rapport du traitement.

ACQUISE est cette augmentation ou obtention de résistance d'une plante active en rapport du traitement.

SENSIBILITÉ: Aptitude d'une plante de montrer des symptômes de maladie.

INSENSIBILITÉ: Capacité d'une plante de subir le développement ou (et) la multiplication d'un parasite ou virus ou l'action d'une autre cause pathogène sans montrer des symptômes de maladie.

TOLÉRANCE: Capacité d'une plante de subir le développement ou (et) la multiplication d'un parasite ou virus ou l'action d'une autre cause pathogène sans faire paraître des symptômes évidents de maladie.

NÉCROSE AVORTOGÈNE: La mort subite du tissu au point d'attaque du parasite ou virus arrêtant la marche du pathogène.

Report of the Temporary Committee on Fungous Nomenclature. In the past summer the President of the Society appointed a committee on plant pathology for the purpose of cooperating with a similar committee of the British Mycological Society. The purpose involved was twofold: first, to work toward a stabilized nomenclature for the fungi, particularly for those species involved as plant pathogens, and secondly, to consider again after the lapse of some years the possibility of the standardization of common names of plant diseases. The British committee has made notable progress along both lines through concrete proposals for *nomina generica conservanda* (see Trans. Brit. Myc. Soc. 23: 215-232, 281-292. 1939) and in the publication of a list of common names of British plant diseases. This list is now in its second edition, and a third has been in preparation. Both the common names employed in this list and the corresponding Latin names of the pathogens have had wide acceptance throughout the British Empire.

The American committee is giving careful thought to both phases of the problem. As a preliminary step, the proposal of the British committee for the conservation of *Urocystis* versus *Tubercinia* will be seconded by a note in PHYTOPATHOLOGY. Further consideration will be given to other genera for conservation in accordance with the International Rules of Botanical Nomenclature, and it is hoped a definite beginning can be made on the admittedly difficult task of arriving at a generally acceptable series of common names for American plant diseases.

G. L. ZUNDEL, Chairman, J. A. STEVENSON,
C. M. TUCKER, D. S. WELCH, ERDMAN WEST

Report of Temporary Committee on Publicity. Your temporary committee on publicity wishes to report that it finds immense possibilities of popularizing phytopathology and improving its public relations. To accomplish this the committee should function throughout the year. It is only by the sincere and unselfish cooperation of the members that these relations can be suitably maintained.

The committee can act only as a clearing house of information furnished by the pathologists of the country through the committee to the science writers, reporters, and magazines. It is not the purpose of the committee to replace any local publicity, but rather to further national distribution of new information and correlate this information with plant pathologists, giving full credit to the author.

We suggest that the committee be made permanent.

C. T. GREGORY, Chairman, J. A. PINCKARD, A. J. ULLSTRUP,
J. H. JENSEN, H. W. RANKIN, W. S. SNYDER

Proposed Policies and Functions of the 1940 Standing Committee on Publicity and Public Relations. It is the sentiment of the Society that plant-pathological problems are not receiving their proper recognition in the press and that this lack of publicity is seriously affecting the recognition and support of our work. A committee of 10 members was appointed to study this problem and to improve our publicity relations.

It is not the function of this committee to interfere in any way with the local or national publicity arrangements that may already be extant, but rather to serve as a tool to further implement our publicity. Its success depends solely on the whole-hearted cooperation of the pathologists of the country.

The functions and policies of the committee should be:

1. To serve as a contact agent between pathologists and the various news agencies to the end that plant pathological news items may be given wide publicity.
2. To create and maintain public interest in plant diseases and their control by showing that crop losses caused by these diseases may affect both farm and city people and by keeping the people informed of the latest developments in control.
3. To give full credit to the investigator or source of the information.
4. To release such publicity immediately after it has been published in a scientific journal, unless specifically permitted by the author to release the information immediately upon its receipt.
5. To issue dignified, factual news reports to accredited science-news writers and to suppress inaccuracies and sensationalism through indiscriminate releases to unknown and

untried science writers. It is not the committee's intention to release news items to local papers but to the national chains that employ the very best science writers.

C. T. GREGORY, Chairman, O. C. BOYD, J. H. JENSEN, J. A. PINKARD, G. H. STARR, FRANK MCWHORTER, A. G. NEWHALL, A. J. ULLSTRUP, G. F. WEBER, P. A. YOUNG

Summary of Report of Committee on Proposed Cumulated Index for PHYTOPATHOLOGY. It is recommended that the Council of The American Phytopathological Society authorize the preparation and publication of a cumulated index covering the first 30 volumes of PHYTOPATHOLOGY, and it is suggested that the program be financed by funds to be raised by the Committee on Donations and Legacies, with eventual reimbursement, at least in part, from receipts on the sale of the index, unless a better plan be forthcoming.

A statistical study of the first 28 volumes of PHYTOPATHOLOGY indicated an average of 20 index pages per volume, with 9 titles covered per index page. This would work out to 600 pages for the 30 volumes. However, due to much combining of entries in a cumulated index, it is estimated that the 30-volume index would not run over 400 pages, and very probably it would be much less, using the general type of indexing employed in the current volume.

It is recommended that qualified pathologists be invited to index one volume each at an honorarium of \$25.00 for 12 issues (2 volumes for the earlier years, and 1 volume beginning with 1918), the index entries to be typed on perforated sheets to be furnished them, along with directions for indexing.

It is estimated that the total cost of preparing the index for the printer would not exceed \$1,000.00, including honoraria, correspondence, index paper, and clerical work (alphabetizing and typing). The printer's bill, depending on the paper stock used and the number of pages, should not exceed \$2,100.00 to \$2,400.00 (including mail). An edition of 1,500 is suggested. A tentative quotation as to price for pre- and post-publication orders, respectively, might be \$3.50 and \$3.75 to members, and \$3.75 and \$4.00 to non-members, but perhaps higher figures and a wider spread in price would be more desirable.

If approved, (1) work should go forward immediately, and (2) an announcement should appear in PHYTOPATHOLOGY. The Committee is not in complete agreement as to the advisability of circularizing the membership at this stage for pledges to purchase the Index when published. Which step should precede is probably a matter for the Council to decide.

The full report of the Committee follows:

Report of Committee on Proposed Cumulated Index for Phytopathology. In the spring of 1939, preliminary to any discussions or actions by this Committee, a study was made of the 28 volumes of PHYTOPATHOLOGY then completed, counting the pages of text and of index, numbers of papers, abstracts, reviews, and notes, the total number of titles (sum of last four items) indexed, number of titles per index page, and average number of text pages per title for each of these volumes. These data are summarized in the tabulation on page 370.

Miss Bien of the Department Library indexed vol. 26, and the Chairman of this committee, vols. 27 and 28: It will be noted that almost exactly the same amount of index space per title was used in these three volumes, although the indexing system for vol. 26 differed from that of vols. 27 and 28. Preceding volumes were indexed by parcelling out the individual issues to different pathologists and librarians, so that much variation occurred and was to be expected. Furthermore, the number of entries required per paper depends to a large extent on the character of the contribution—more often than on its length: e.g., the published abstracts require almost and sometimes quite as much indexing space as the full papers; and papers with many organisms or new hosts take a correspondingly greater space to index than those concerned with one or only a few phases of a disease of one host. After looking over some of the extremes in index space used it was concluded that perhaps the closest estimate might be obtained by finding the average for the 28 volumes: this figure comes out as slightly over 9 titles per index page, while the average of index pages per volume is 20.2 (the first 7 volumes had only 6 issues each; there were 567 index pages in the 28 volumes, and 5217 titles—exclusive of errata and Society reports—to be indexed). Allowing 9.2 titles per index page for the 5217 titles we get a total of 567 pages for the 28 volumes, or 20.2 index pages per volume: for 30 volumes this would become 606 pages. In a cumulated index there is a good deal of combining of entries, so it is believed safe to assume that the 30-volume index would not take over 400 pages and very probably less.

A number of individuals agree that the cost of hiring a professional indexer would be prohibitive, and that a professional indexer—unless at the same time a plant pathologist—would do an inadequate job. On the basis of 11 years' experience as associate editor of Biological Abstracts, including a hand in working out the index system used and in training indexers, the chairman fully believes that with a carefully

PHYTOPATHOLOGY

Vol.	Year	Pages text	Pages index	Number of				Titles indexed	Titles per index page	Av. pp. per title	No. of issues
				Papers	Absts.	Reviews	Notes				
1	1911	204	4	37	17	5	59	14.7	3.4	6 ↓
2	1912	276	11	41	37	4	9	91	8.3	3.0	
3	1913	313	13	44	31	4	17	96	7.4	3.2	
4	1914	417	16	35	100	2	21	158	9.9	2.7	
5	1915	356	9	52	20	1	15	88	9.9	4.0	
6	1916	454	10	47	69	5	33	154	15.4	2.9	
7	1917	458	11	49	63	4	28	144	13.1	3.2	
8	1918	627	14	59	7	36	92	6.6	6.8	12 ↓
9	1919	587	11	55	19	74	6.7	7.9	
10	1920	554	12	57	61	2	19	139	11.6	4.0	
11	1921	516	20	69	98	1	42	210	10.5	2.4	
12	1922	585	15	70	147	...	15	232	15.5	2.5	
13	1923	562	22	69	100	...	22	191	8.7	2.9	
14	1924	588	21	61	150	...	26	237	11.3	2.5	
15	1925	809	19	86	84	3	21	194	10.2	4.6	
16	1926	1012	19	78	95	4	23	200	10.5	5.0	
17	1927	836	21	79	65	3	12	159	7.6	5.2	
18	1928	1030	27	75	115	3	25	218	8.0	4.7	
19	1929	1147	26	88	95	2	55	240	9.2	4.7	
20	1930	1011	21	89	117	4	33	243	11.6	4.1	
21	1931	1207	22	90	96	6	21	213	9.7	5.6	
22	1932	1002	29	74	105	5	37	221	7.6	4.5	
23	1933	1006	28	78	120	7	38	243	8.7	4.1	
24	1934	1318	52	97	118	9	72	296	5.7	4.4	
25	1935	1118	43	81	165	6	36	288	6.7	3.9	
26	1936	1160	22	93	91	7	37	228	10.3	5.1 CBn	
27	1937	1186	21	88	88	5	36	217	10.3	5.4 FVR	
28	1938	939	28	99	139	7	47	292	10.4	3.2 FVR	

worked out set of directions—including examples—and a judicious selection of invited personnel we can enlist pathologists to do a volume or two each (the early 6-issue volumes each to count as half a volume). After 3 years' indexing on PHYTOPATHOLOGY, it is estimated that each issue would average 4 or 5 hours. Again, taking the experience of Biological Abstracts and Chemical Abstracts, it is believed that an honorarium of \$25.00 per each 12 issues would do the trick. For 50 to 60 hours' work \$25.00 may seem rather small pay for scientifically trained people, BUT the experience of these two journals has proved that an honorarium, however inadequate, serves as the added impetus needed to activate a person who is already interested in the subject. If the analytical-type of subject index used by Biological Abstracts—and the one used in PHYTOPATHOLOGY now for the third year—is employed it will be necessary to index anew only through volume 26, the index cards beginning with vol. 27 having been saved. Counting two of the 6-issue volumes as one, this leaves 22.5 "volumes" to be indexed: at \$25.00 each, this would come to \$562.50.

For 13 years, Biological Abstracts has been using perforated sheets for typing the index entries. These are easy to type and handle, can be edited against the original papers readily before tearing apart into the individual 10 cards each, can be easily and rapidly marked for the printer and sent without copying, if so desired (thus making for accuracy), and there has never been any trouble with regard to printers' accepting them as copy. All the Botanical Abstracts and Biological Abstracts indexes have been sent in this way, and several other printers, including the Government Printing Office, have accepted this system without question. By numbering in final alphabetized order with a numbering machine assurance may be had that none of the entries are skipped. Biological Abstracts has never numbered them and, so far as we know, no entries have been lost.

This is probably not the time for a detailed discussion of indexing policies, but the general analytical type used in Biological Abstracts and in the last three volumes of PHYTOPATHOLOGY is preferred by the Committee; main subject words are alphabetized flush with the margin, and each subentry is inset 1, 2, or 3 places from the margin,

respectively. This form is most easily used, enabling the reader to see at a glance what is there. It may take somewhat more space than the "block system" formerly used in PHYTOPATHOLOGY, but this is more than made up for by the change in the author index and reduction of entries to simplest terms. Through volume 26 the entire titles of papers followed the authors' names in the index: this seemed unnecessary, since subject matter is all given first-place entry in the index. In the current index each author is given only first-place entry, joint authorship being indicated by the page reference in "(—)". Thus, John Doe and Henry Brown occur in the index as: "Doe, John, 22"; "Brown, Henry, (22)". New taxonomy, as in the past, is in blackface type.

Should the Cumulated Index program be approved, a detailed set of directions will be worked out and distributed to the invited collaborators doing the indexing. In the current volume, the Chairman has endeavored to follow the general lines approved by this Committee. However, since the Society has endeavored recently to enlist other than professional phytopathologists in subscribing to its journal, an effort has been made to present the material in such manner as to make unnecessary any knowledge of scientific names, though the latter also have been included. In a cumulated index it would be less readily feasible to go to quite this detail. All such matters will, of course, receive full consideration when or if the project is approved.

The Committee's estimates on the cost of *preparing* the proposed 30-volume index for the printer follow:

Honorarium at \$25.00 per each 12 issues to the indexers (index cards of Chairman's indexing saved for vols. 27-29, and will be also for 30) through 1936	\$562.50
Alphabetizing cards and typing	100.00
Honorarium for final editing (at least a two-months' full-time job)	200.00
Correspondence (postage on the index included in printer's estimates)	25.00
Index paper at \$2.50 per 1000 in 5000 lots, like sample (5000 should be ample, with margin of safety)	12.50
Total	\$900.00

With an added \$100.00, making it an even \$1000, for safety, the total cost would then be this amount plus the printer's estimates, which follow:

"The per-page price can stand exactly as it is. If you have more or less pages an adjustment can be made, or if we see the copy we shall be glad to make a bid on the whole manuscript exactly as it is submitted to us. We, of course, shall be glad to bill the Society on the per-page basis according to how many pages the manuscript may run to. This seems like a more satisfactory way of handling it, but we can make an estimate and abide by it, though we would then have to allow a margin of safety, but if it is done on the per-page basis there is no gamble on either side.

"Based on the usual index in *Phytopathology* we estimate the total price would be as follows:

	1,000 Copies	1,900 Copies	Weight each
Phytopathology stock	\$2,226.25	\$2,600.81	2½ lbs.
Science stock	2,092.00	2,347.81	2 lbs.
Biological Abstracts stock	2,107.50	2,377.31	1½ lbs.

(Book rate—1½ cts. per lb. in U. S. A.)

"You will note above that we have also given the weight of each book. Two-and-a-quarter lbs. would cost 4½ cts. to mail, while the Biological Abstracts paper would only cost 1½ cts. However, the penalty charge on thin paper would be a great deal more than the increased postage charge based on the weight of the books. As long as the book rate is in effect I think it is best that you use paper which is used in *Science*, sample of which you will find enclosed. We are in a position to give you this paper at a somewhat reduced cost because we buy it in large quantities for *Science* and other publications. We are giving you on a separate sheet the details showing how we arrived at the total cost." [Excerpts from letter of November 30, 1939, from Jaques Cattell, Vice-President and Secretary, The Science Press Printing Company.]

As above detailed, the estimates are for editions of 1000 and 1900, respectively; perhaps an intermediate number might more nearly fit the case. The Committee favors an edition of 1500 and the paper stock used in *Science*. A suggested price to Society members might be tentatively set at \$3.50 for prepublication and \$3.75 for post-publication orders, with \$3.75 and \$4.00, respectively, as the prices to nonmembers, though higher figures and a wider price spread might be desirable. Possibly announcement of exact prices and paper stock should be deferred until later.

If the green light is given by the Council, indexing work on the published volumes of PHYTOPATHOLOGY should go forward immediately after January 1, 1940, in order that the index cards for the first 29 volumes may be prepared, alphabetized, and edited so far as possible before the end of the year. During the year, as the issues appear, the

indexing for volume 30 can be done in duplicate for use (1) in the 1940 current volume index and (2) for interpolation in the cumulated index file as the work goes on. In this way the completed Cumulated Index should be ready to go to the printer in January, 1941; the date of publication after that would depend on promptness of printer and proofreaders.

The Committee agrees that Society members and subscribers should be circularized by double post card (with blank order or pledge to be signed on the return part), and that a full-page announcement on Cumulated Index plans be published in PHYTOPATHOLOGY at an early date. The only question is whether the *circularization* should be done immediately on approval of the project, or should await partial fulfillment of the work, when the number of pages, costs involved, and prices to be charged can be more definitely estimated.

The Committee feels that the advancing of necessary funds for carrying out the preparation of this index might well be placed in the hands of the Committee on Donations and Legacies of the Society. It is believed that such use would be entirely within the original objectives of the Lyman Fund, and that such use of any other funds in the hands of this Committee would be entirely proper and at the same time furnish a good example to the Society of the value of having such moneys available for its use. It is, therefore, suggested that the Council take up this matter with the Committee on Donations and Legacies and if agreeable to all concerned that this Committee be authorized to have charge of financing the Cumulated Index project, to be reimbursed by moneys received from the sale of the Index.

At this point it will probably have been surmised that the Committee recommends the preparation of a Cumulated Index for the first 30 volumes of PHYTOPATHOLOGY, and such is the case.

Respectfully submitted to the Council of the American Phytopathological Society.

FREDERICK V. RAND, Chairman

GEO. L. PELTIER

NEIL E. STEVENS

Supplement

Printer's Estimate on the Cost of Printing PHYTOPATHOLOGY Index

1,000 Copies, 8 pt. on 8, per page	\$ 5.48
1,900 Copies, 8 pt. on 8, per page	6.35
Additional 1,000 Copies, per page97
1,000 Covers and Covering	24.25
1,900 Covers and Covering	41.80
Additional 1,000 Covers and Covering	19.50

Cost of Printing 1,000 Copies, 400-page Index (PHYTOPATHOLOGY Stock):

400 pp. 8 on 8 at \$5.48	\$2,192.00
Covers and Covering	24.25
Mailing at \$10.00 M	10.00

	\$2,226.25
Price Using <i>Science</i> Stock	2,092.00
Price Using Stock similar to <i>Biological Abstracts</i>	2,107.50

Cost of Printing 1,900 Copies, 400-page Index (PHYTOPATHOLOGY Stock):

400 pp. 8 on 8 at \$6.35	\$2,540.00
Covers and Covering	41.80
Mailing at \$10.00 M	19.00

	\$2,600.80
Price Using <i>Science</i> Stock	2,347.81
Price Using Stock similar to <i>Biological Abstracts</i>	2,377.31

The Sixth Pacific Science Congress. The Sixth Pacific Science Congress was held at Berkeley, Stanford University, and San Francisco, California, July 24 to August 12, 1939. For the first time in the history of these congresses, plant pathology was represented by a definite subsectional program (Section VI, Botany, Subsection VI-B, Phytopathology). The program was organized by E. C. Stakman, Chairman. The meetings were held at the University of California, Berkeley, on August 4 and 5. The general topics were Virus Diseases of Plants, Variation in Plant Pathogens, Dissemination and Distribution of Plant Pathogens, and Plant Diseases Caused by Nutrient Deficiencies. Among those who contributed to the programs and discussions were Eubanks Carsner,

G. O. Oefemia (University of the Philippines), T. E. Rawlins, J. M. Wallace, C. W. Bennett, W. C. Snyder, H. N. Hansen, J. H. Craigie (Winnipeg), José Vallega (Argentina), Hidenhumi Asuyama (Tokyo Imperial University), H. S. Fawcett, H. R. McLarty (Summerland, B. C.), Kenneth Smith and V. H. Blackman (Cambridge, England), and H. L. Lyon (Honolulu). A total of 43 were in attendance.

At the close of the session on Virus Diseases of Plants, Eubanks Carsner, Chairman, the following resolution directed to the International Committee on Virus Nomenclature was adopted:

This Section favors the use in any system of plant-virus nomenclature of the Latin generic name of the host rather than the vernacular name, and the omission of the specific name of the host.

Report of the Representative on the International Union of Biological Sciences. Conditions in Europe have caused postponement of the scheduled 1940 meeting of the Union of Biological Sciences, which was to have been held at Stockholm in July. At this date, no prediction can be made as to when this meeting will take place. No proposals have been received by your representative the past year either from officers, committees, or individuals of our Society.

A. G. NEWHALL

Report of the Delegates of the Congress for Microbiology Held in New York, N. Y., September 2 to 9, 1939. This was the first Congress for Microbiology in which phytopathologists and mycologists were given an opportunity to meet pathologists and mycologists specializing in diseases of animals and human beings. Section VI (Fungi and Fungous Diseases) was permitted to hold five full morning programs and three joint programs with other Sections. While many of the 112 papers scheduled in these eight programs dealt with purely microbiological and physiological subjects, nevertheless they were all of fundamental importance to plant pathologists. Section III (Virus and Viral Diseases) scheduled 124 papers, many of which were of marked importance to phytopathologists.

In the symposium on Host-Parasite Relationships, dealing with (1) Etiology and Pathogenesis, (2) Tissue Reactions, and (3) Natural Resistance Including Immunity, G. H. Coons, J. C. Walker, and E. C. Stakman discussed the topics as plant pathologists, while Drs. Fred D. Weidman, D. J. Davis and J. G. Hopkins discussed them from the standpoint of medical pathology.

A. J. Riker cooperated with Michael Levine in organizing the program of 12 papers on the "Effects of Microorganisms and Chemicals on Atypical Growth in Plants."

A joint program was held with Section I (Variation and Taxonomy) on "Classification of Actinomycetes and Higher Fungi."

Professor L. R. Jones, as Dean of American Phytopathologists, was elected one of the five honorary presidents of the Congress, the only one from the United States. E. C. Stakman, C. L. Shear and A. H. Reginald Buller were elected Vice-Presidents of Section VI. G. M. Reed and Michael Levine served on the local committee of arrangements for Section VI and other members assisted. Sixteen hundred persons registered officially at the Congress. The Proceedings will include the addresses given at the general sessions of the Congress, published in full, and abstracts of over six hundred papers presented on the programs.

B. O. Dodge served on the Executive Committee of the Congress and as Convener of Section VI (Fungi and Fungous Diseases). He addressed a general session on "Some Problems in the Genetics of Fungi."

The Fourth International Congress for Microbiology is scheduled to meet in Copenhagen in 1942. It is recommended that the Society take an active part in the Congress.

B. O. DODGE and WM. H. MARTIN

Report of the Resolutions Committee. 1. RESOLVED that The American Phytopathological Society express its appreciation to the A. A. A. S. committees responsible for the arrangements that have contributed so effectively to the success of the 1939 meeting in Columbus.

2. RESOLVED that The American Phytopathological Society convey to the management of the Neil House expression of gratitude for the courteous and efficient service extended to the members attending the thirty-first annual meeting. This resolution was enthusiastically adopted by a rising vote.

3. RESOLVED that, on behalf of The American Phytopathological Society, we express our appreciation to the various local agencies and committees for their many courtesies and most efficient services; to the Governor, the honorable John W. Bricker, for a delightful tea arranged for our guests; to The State Department of Visual Instruction and the Spencer Lens Company for supplying us with projection equipment; to the troop

of Boy Scouts of America for messenger service during the sessions; and to the City School Teachers of Columbus for their help in operating the projectors.

4. RESOLVED that, on behalf of the members of our Society, we express to our officers and committee members our deep appreciation for their untiring efforts in furthering the best interests of our Society.

5. RESOLVED that, on behalf of the Society, we express to our committee on arrangements for the Columbus meeting, W. G. Stover, chairman, A. L. Pierstorff, C. C. Allison, Harry Atwood, H. C. Young, and P. E. Tilford, and A. J. Riker and the subcommittee on exhibits, our gratitude for their very substantial contribution to the success of the meeting, and to the Department of Plant Pathology of the University of West Virginia, particularly Genevieve Clulo, Eldor Martin, William Dorrell, and Donald Hoffmaster, for entertaining us so delightfully during the dinner.

6. RESOLVED that, on behalf of the Society, we express to Professor L. R. Jones our deep appreciation for his thoughtfulness and generosity in donating to the Society five hundred separates of his memoir on the life of Dr. Erwin F. Smith.

G. W. KEITT
M. W. GARDNER
G. H. COONS

ACTION BY THE SOCIETY AT THE 1939 COLUMBUS MEETING

Elections and Appointments. The appointments made, as provided by the Constitution, by the President or the Council since the previous meeting were approved by the Society in business session. The election committee opened and counted the ballots, and the results were announced to the Society. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees. Sixty-two applicants were elected to membership.

The Society confirmed the Council's appointment of the following new members to the Editorial Board of PHYTOPATHOLOGY: J. S. Boyce, C. W. Bennett, and R. P. White.

Reports of Officers, Representatives, and Committees. The reports for the year 1939, as presented on previous pages, were read and accepted.

Recommendation Regarding Abstracts. The Society confirmed the Council's recommendation that the Secretary be allowed to copy the titles of all abstracts before they go to the Editorial Committee and make up the program from that material, so that the program may be in the mail early; and, that the Secretary be notified by the Editorial Committee of any rejections of abstracts. (Papers received after November 1 will be rejected.)

Committee on Nomenclature and Classification of Plant Viruses. The Society confirmed the Council's recommendation that the temporary committee on virus nomenclature be made a standing committee.

Committee on Publicity and Public Relations. The Society confirmed the Council's recommendation that the temporary publicity committee be made a standing committee, Publicity and Public Relations.

Committee on Regulatory Work and Foreign Plant Diseases. The Society confirmed the Council's recommendation that, since at the request of the chairman of the committee that all members of this committee in the United States be dropped, the Committee on Regulatory Work and Foreign Plant Diseases be discharged.

Committee on Research Monographs of the Association of Land-Grant Colleges and Universities. The Society confirmed the Council's recommendation that the incoming President appoint a temporary committee to study and make a report as soon as possible on the request of the committee on Research Monographs of the Association of Land-Grant Colleges and Universities.

Committee on Terminology of Immunology and Use of Technical Words. The Society confirmed the Council's recommendation that the Committee on Terminology of Immunology and Use of Technical Words continue to be temporary, and the report be printed in PHYTOPATHOLOGY in the Annual Report.

Constitution and Standing Rules. The Society confirmed the Council's recommendation of the publication of the Constitution and Standing Rules.

Cumulated Index of PHYTOPATHOLOGY. Upon recommendation of the Council, the Society voted to go on record as endorsing in principle the report of the committee, and instructing the President-elect to make any appointments or changes he deemed necessary for the committee to work out the details of the plan.

Summer Meeting. The Society, on Council recommendation, voted to hold a summer meeting at Seattle, Washington, in connection with the A. A. A. S. summer meeting, June 17-22, 1940, and that arrangements be in charge of the Pacific Coast Division of the Society.

The 1940 Annual Meeting. It was recommended by the Council, and voted by the Society, that the time of the annual meeting in 1940, at Philadelphia, Pennsylvania, be from the morning of December 27 (Friday) to December 31 (Tuesday), with the Sunday intervening to be used for informal conferences.

Council Policy. The Council expressed the desire to state that it is at present the policy of the Council, and they approve of the idea, of changing the appointments on committees often, thus putting them on a rotating basis.

Council Recommendation. The Council reported that the Society has been helping the Central Bureau of Schimmelcultures by giving them advertising space in the journal. In order to help the Bureau financially, the Council urged members to buy cultures from this Bureau as they are needed.

IVAN CLAUDE JAGGER

August 12, 1889–February 16, 1939

Ivan Claude Jagger was graduated from Cornell University in 1911 with the degree of Bachelor of Science in Agriculture, and in 1913 he was granted the degree of Master of Science by the University of Wisconsin.

From 1911 to 1913, Mr. Jagger was an Industrial Fellow, and 1913–14, Instructor in Plant Pathology at Cornell University; from 1914 to 1918 he was Assistant Professor of Plant Pathology in the College of Agriculture, Cornell University, and Instructor in Biology at the University of Rochester; from 1918 to the time of his death he was Pathologist, and in later years Senior Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, except during 1926 when he held a fellowship with the International Education Board.

Mr. Jagger achieved outstanding success along a number of lines, especially in breeding high-quality lettuce and melons resistant to mildews and certain other diseases. He possessed a most pleasing personality, combining thoughtfulness, congeniality, and genuineness. He had many friends and all regarded him most highly.

LAWSON MERRILL HILL

March 28, 1909-May 6, 1939

Lawson Merrill Hill was graduated from West Virginia University in 1935 with the degree of Bachelor of Science in Agriculture, and in 1937 he was granted the degree of Master of Science by the same institution.

From 1936 to the time of his death, he was research Assistant in the Department of Plant Pathology and Bacteriology of the West Virginia Agricultural Experiment Station.

Mr. Hill was a student and investigator of much promise. He was especially interested in the microchemistry of pathological tissues. He was one of the most popular students in his university class and won high honors in many fields of activity. His friendly nature and pleasing sense of humor endeared him to all his associates.

ROLAND ELISHA STONE

November 4, 1881–June 4, 1939

Roland Elisha Stone was graduated from the University of Nebraska in 1906 with the degree of B.Sc. In 1908 he received his M.Sc. from Alabama Polytechnic Institute and his Ph.D. was conferred by Cornell University in 1913. At Cornell University he was also elected a member of the Sigma Xi.

From 1912 until 1917, Dr. Stone was Lecturer in Botany at the Ontario Agricultural College, Guelph, Canada, and in 1917 he became Associate Professor of Botany, which position he held until his death. He spent twenty-seven years in the service of the Ontario Agricultural College.

He was a sustaining life member of The American Phytopathological Society, a member of the Canadian Phytopathological Society, the Botanical Society of America and a Fellow of the American Association for the Advancement of Science.

Dr. Stone's chief interests were in teaching, in which he excelled, and in research in the field of plant pathology and mycology. Among his many achievements were the selection of varieties of canning peas resistant to root rot and suitable to the Ontario Canning Industry, and his publications on the edible and poisonous mushrooms of Ontario.

He was an enthusiastic botanist with high ideals, a keen sense of duty and the ability to inspire and direct students in their search for scientific knowledge. He had a quiet unobtrusive manner and a kindly personality combined with a dry sense of humour. These qualities endeared him to all who became well acquainted with him. His outstanding characteristic, however, was his spirit of loyalty to his profession, his College, his colleagues, and his friends.

EDWARD JACOB PETRY

June 24, 1880–October 8, 1939

Edward Jacob Petry was graduated from Ohio State University, with the degree of Bachelor of Science, in 1907. In 1914, he received the degree of Master of Science from Purdue University, and in 1925, the degree of Doctor of Philosophy from Michigan State College.

From 1907 to 1910, he served as Assistant in Botany, Cornell University; from 1911 to 1916, he was Instructor in Agronomy, and 1916 to 1918, Assistant Professor of Agricultural Botany, Purdue University; from 1918 to 1920, Instructor in Botany, University of Michigan; from 1920 to 1923, Professor of Botany, South Dakota State College; from 1923–1924, Consulting Botanist, South Dakota Experiment Station; 1924–1925, Survey Botanist, South Dakota Geological and Biological Survey; from 1926 to 1929, Professor of Biology, Hendrix College; from 1929 to 1931, Professor of Biology, Central College (Mo.); from 1931 to 1933, Professor of Botany and Associate in Physiology, Coe College; from 1933 to 1935, Consulting Biologist and Chemist, Cedar Rapids Water Works; 1936, Chemist, Consumers Co-operative Association, Kansas City, Mo.; 1937 to time of his death, Paint Chemist, Ebony Paint Co., Kansas City, Mo.; also, 1939 to time of his death, Professor of Chemistry and Medical Technologist, Central College of Osteopathy, Kansas City, Mo.

Dr. Petry was a life member of The American Phytopathological Society and had been a member of a number of other scientific societies. He had a naturally inquiring mind and was always anxious to ferret out the unknown in any situation. He had a very friendly disposition, and aimed always to be helpful to those in distress and to practice the "Golden Rule."

ERRATA, VOLUME 29

Page 75, table 2, column 1, *read* Feb. 10 *for* Feb. 11; column 4, *read* Feb. 11 *for* Feb. 10.

Page 92, line 12, *read* sedimented less rapidly *for* sedimented more rapidly.

Page 658, line 12, *read* $\frac{p}{1-p}$ *for* $\frac{1}{1-p}$.

Page 908, line 8, last paragraph, *read* hood *for* blood.

PRELIMINARY ANNOUNCEMENT

The American Phytopathological Society will hold its summer meeting at the University of Washington, Seattle, Washington, June 19-22, in connection with the regular meetings of the Pacific Division of the Society.

The program will include a general meeting of horticulturists, entomologists, and plant pathologists, a symposium on fruit tree viroous diseases, an all-day field trip to the Western Washington Experiment Station at Puyallup, and three half-days for the presentation of papers.

Plant pathologists who wish to present papers at this meeting should send their titles, prior to May 1, to L. D. Leach, Secretary-Treasurer, Pacific Division, Davis, California.

CULTURAL AND GENETIC STUDIES ON *USTILAGO ZEAE*¹

C. G. SCHMITT

(Accepted for publication January 5, 1940)

INTRODUCTION

These studies were initiated to determine the nature of inheritance of factors for characters in *Ustilago zea* (Beckm.) Unger, and to arrive at a better understanding of the phenomenon of sectoring in culture. Inbreeding was undertaken to establish uniform lines with distinctive characters satisfactory for crossing and favorable for the study of induced mutation.

Ustilago zea is a desirable species for use in the study of the genetics of fungi because its life cycle is relatively short (as many as 10 generations may be grown in a single year); it is a heterothallic species with distinct haploid characters; each chromatid can be sampled; and the fungus is widely distributed, available in quantity, and readily cultured.

METHODS OF SINGLE-SPORE ISOLATION AND OF INBREEDING

Monosporidial lines were established by isolating sporidia from promycelia with a modified Chamber's micro-manipulator. Dickinson's (7) and Hanna's (13) techniques were followed with the exception that the sporidia were transferred immediately to agar slopes by means of a flat-tip transfer needle. Germination of chlamydospores occurred in from 12 to 24 hours on Czapek's agar at 25–30° C. At lower temperatures germination was delayed and a typical promycelium was not always formed. These findings confirm those of Hüttig (16). The spores exhibited no period of dormancy. High germination was obtained from spores removed from galls that were not entirely dry. A discussion of the literature on dormancy of spores of this species has been presented by Stakman (24).

The system of culture numbering employed by Christensen (4) was used. Inbreeding was practiced for 10 successive generations by introducing by hypodermic needle all possible combinations of cultures of monosporidial lines from the same promycelium in pairs into susceptible corn plants. Ten plants usually were inoculated with each cross. Cultures of individual lines also were inoculated into plants to determine the extent of monopathogenicity in the isolates. In preliminary tests, plants of all ages were inoculated. It was found that infection occurred when the sporidia were in contact with meristematic tissue, irrespective of the age of the plants. Plants in the greenhouse were inoculated when a week old, and mature galls were harvested 3 to 4 weeks later. The greenhouse temperature was held at 27–32 degrees C., because it was found that gall formation occurred within this range but did not occur below 21° C. Tisdale and Johnston (27) found that seedlings in the greenhouse were as susceptible

¹ A portion of a thesis submitted to the Department of Botany, University of Missouri, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

to infection as were older plants. They emphasized the necessity of taking cognizance of the relation of temperature to infection. Plants grown at low temperatures inoculated with compatible lines usually developed anthocyanin at the point of inoculation. This phenomenon was not observed in plants inoculated with incompatible lines.

MEDIA

During the first 3 generations of inbreeding the following media were used for comparison of cultural characteristics: Modified Czapek's (MgSO_4 , 0.30 g.; KH_2PO_4 , 1.25 g.; KCl , 0.50 g.; FeSO_4 , 0.01 g.; Asparagine, 1.00 g.; NaNO_3 , 0.50 g.; sucrose, 30.00 g.; agar, 15.00 g.; and distilled water to 1 liter), malt-extract agar, Carter's, potato dextrose, Difco corn-meal agar, and a medium (referred to in this paper as "S"), used by Stakman *et al.* (25), which consisted of: sucrose, 3%; $\text{Ca}(\text{NO}_3)_2$, 0.006%; and agar, 1.8%. Subsequent generations were grown upon Difco corn-meal agar. To compare the cultural characters of the first generation with those of any succeeding generation, the lines to be compared were inoculated on a given date into 125 ml. flasks containing 35 ml. of the same lot of medium. By following this procedure the fluctuations in characteristics of a line that might result from slight variations in the constitution of 2 separately prepared lots of a given medium were avoided. Cultures were then incubated at 25–27° C. Stock cultures were maintained on modified Czapek's agar in 6-inch test tubes at 10–18° C. to prevent sectoring.

EFFECT OF THE MEDIUM ON SECTORING

The effect of the medium on the rate of sectoring of this fungus has been studied in some detail by Stakman *et al.* (25). They found that the kind of medium used affected mutation rate. Table 1 summarizes the influence of

TABLE 1.—*Effect of the medium on rate of sectoring in Ustilago zeae. Twenty monosporeidial lines were grown in duplicate on each medium. Results were recorded following 3 weeks' incubation at 25–27° C.*

Medium	Number of sectors
Difco corn-meal agar	68
Modified Czapek's agar	79
Malt-extract agar	90
S	94
Potato-dextrose agar	106
Carter's medium	107

the media on frequency of sectoring. On the basis of these results, modified Czapek's was selected as a desirable medium for the maintenance of stock cultures, since the rate of sectoring on it was relatively low and the rate of growth good. Difco corn-meal agar was selected as the medium to detect mutation following irradiation because of its uniformity and the low rate of sectoring exhibited upon it.

When a given line of this fungus is grown on a medium of one composi-

tion, it may exhibit an appearance at variance with that displayed when it is grown on another medium, but if transferred from the second medium back to the first the appearance of the colony will be identical with that originally shown on the first medium. No gradual change in type has been observed in any case. These observations are in conformity with those of Stakman *et al.* (25).

EFFECT OF IRRADIATION ON SECTORING

That ultraviolet radiation is sometimes a means of inducing mutation in fungi has been demonstrated by a number of investigators. Stevens (26) reported ultraviolet radiation effective in inducing perithecial formation in *Glomerella cingulata* (Stoneman) Spaulding and Schrenk and pycnidial formation in *Coniothyrium* sp. Greaney and Machacek (12) produced a white, fertile saltant of *Helminthosporium sativum* P. K. and B. by ultraviolet treatment. Hollaender and Emmons (15) and Emmons and Hollaender (10) produced variants in *Trichophyton mentagrophytes* (Robin.) Sab. with monochromatic ultraviolet radiation. The new type colonies of this fungus differed from the normal in form, color, growth rate, and spore production. The wave lengths most effective in killing, 2650 to 2537 Å, also were most effective in production of variants. Dickson (8) found that mutations induced in *Chaetomium cochliodes* by X-rays did not differ essentially in their characters from those induced by ultraviolet treatment. Seven other species of fungi did not respond to irradiation when subjected to the same treatment. He induced saltants in both plus and minus strains of *Phycomyces blakesleanus* Burgeff by exposing them to X-rays.

Twenty-four-hour-old broth cultures of sporidia to be irradiated with ultraviolet or X-rays were spread in a single layer on the surface of Czapek's agar on double-width micro slides, according to Landen's (19) method. Ultraviolet treatments were applied to sporidia of the 5th inbred generation using the 2537 Å line and dosages ranging from 2500 to 6800 ergs/mm². The results are summarized in table 2.

TABLE 2.—Comparison of rate of sectoring in colonies from sporidia irradiated with ultraviolet light (2537 Å) of various dosages. Results after 5 weeks' incubation on Difco corn-meal agar

Line	75: 2 (sporidial)			75: 3a (mycelial)		
Dosage ergs/mm ² .	Total no. colonies	No. of colonies with sectors	Percentage of colonies with sectors	No. of colonies	No. of colonies with sectors	Percentage of colonies with sectors
0	147	107	72.8	134	28	20.9
2,500	60	52	86.7	63	15	23.8
4,000	99	71	71.7	50	9	18.0
5,000	245	177	72.2	131	54	41.2
6,000	39	23	59.0	83	26	31.3

From 2 days to a week after treatment the colonies developing from these treated sporidia were transferred to 125 ml. culture flasks containing

Difco corn-meal agar. Although over 770 such colonies were observed for five weeks following transfer, the rate of sectoring in the colonies from treated sporidia was not in excess of that for the check. The mutation rate apparently was not affected by ultraviolet treatment.

Monochromatic irradiation was applied² to 5th inbred generation chlamydospores, using the 2650 Å line and dosages ranging from 21,000 to 56,000 ergs/mm². There was a noticeable delay in germination of treated spores and the delay increased with the dosage applied. Those given 56,000 ergs/mm². germinated 60 hours after transfer to media. The lethal effect also increased with the dosage. Over 40 sets of sporidia were removed from treated spores, but no indication of mutation induced by radiation was found in the colonies that developed from these sporidia.

The highest X-ray dosage used was approximately 4500 r units. This was not sufficient to exhibit any lethal effect. Only 150 colonies from treated sporidia were observed, but there was no indication of mutation in them.

Failure of irradiation to affect the mutation rate of this fungus strengthens the conclusion drawn by others; *e.g.*, Heldmaier (14), Matsuura (21), Leonian (20), and Christensen and Graham (5), that unstable species are not necessarily more unstable following irradiation. Borzini (3) treated spores of *Ustilago zeae* with ultraviolet after germination was initiated. The promycelia and sporidia produced were shorter and thicker than those of the checks. Other than this no apparent changes were produced.

EFFECT OF TEMPERATURE ON SECTORING AND GROWTH

Some workers have been successful in producing mutations by applications of heat to fungi. Barnes (1) found that exposures of the spores of *Eurotium herbariorum* (Wigg.) Link. for two minutes at temperatures of 60–80° C. produced mutation. In a later work (2) similar results were obtained with *Botrytis cinerea*. Dickson (8) applied heat treatments near the maximum to *Chaetomium cochliodes* but noted no mutants as a result of the treatment.

In an attempt to induce mutation by heat treatments near the thermal death point 12-hour old sporidial suspensions of a tenth-generation inbred line in malt extract broth were held at temperatures of 50, 55, 60, and 65° C. for 10 minutes. Poured plates were then made of the suspensions. The thermal death point was found to be somewhere between 55° and 60° C. Two days later individual colonies were removed from the 55° plates and transferred to agar slants. Although approximately 200 colonies developing from these sporidia were observed on agar plates, only 16 were removed for more extended observation. There was no indication of induced mutation.

The effects of temperature on the rate of growth and sectoring are presented in table 3. The fifth-inbred-generation lines used in this test were 75:2, a sporidial line, and 75:3a, a mycelial line. The results indicate that

² The writer is grateful for technical assistance to E. W. Landen of the Department of Physics of the University of Missouri.

the rate of sectoring is not necessarily highest at the optimum-growth temperature. In general, it may be stated that mycelial lines grow more rapidly than do sporidial lines at temperatures near and below the optimum. Stakman *et al.* (25) likewise found considerable variability between lines in their response to temperature.

TABLE 3.—*The effect of various temperatures upon the growth and rate of sectoring of inbred lines 75:2 and 75:3a of Ustilago zea, after 4 weeks' of incubation. Cultures grown in quadruplicate*

Temperature	<i>U. zea</i>	75:2 (sporidial)		75:3a (mycelial)	
	No. of culture	Average diameter of colony	No. of sectors	Average diameter of colony	No. of sectors
°C.		mm.		mm.	
10	1	10	0	16	0
	2	9	0	17	0
	3	8	0	17	0
	4	8	0	16	0
15	1	13	0	35	0
	2	13	0	33	0
	3	12	0	32	0
	4	12	0	33	1
20	1	18	6	42	0
	2	21	5	41	0
	3	18	3	42	0
	4	15	7	42	0
25	1	21	14	43	0
	2	22	5	46	0
	3	19	10	47	0
	4	20	12	46	0
30	1	22	3	38	2
	2	20	5	38	3
	3	21	2	37	1
	4	23	0	37	0
34	1	20	0	15	0
	2	19	0	9	1
	3	19	0	13	0
	4	19	0	9	0
37.5	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0

EFFECT OF INBREEDING ON SECTORING

To compare the rate of sectoring of lines of the fungus that had been inbred to various generations, quadruplicate cultures of a number of lines were set up on corn-meal agar at a temperature of 25–28° C. (Table 4). Inbreeding failed to increase the stability of this stock of *Ustilago zea*. There was a clear cut difference between the rate of sectoring of sporidial and mycelial lines but in each type the rate of sectoring appeared to be constant.

TABLE 4.—*The effect of inbreeding on the rate of sectoring in Ustilago zeae after 5 weeks' incubation at 25 to 28 degrees C.*

Sporidial lines				Mycelial lines			
No. of spore	No. of gen. inbred	No. of sporidium	No. of sectors	No. of spore	No. of gen. inbred	No. of sporidium	No. of sectors
500	0	1	15	500	0	2a.4	6
		2	18	700	0	4e.1	5
		3	24			1.1	7
		4	29			3e.4	3
700	0	4c.3	20	503	1	1.2	4
503	1	1	21	707	1	1.1	2
707	1	4	17			2.1	6
		1	26			3.1	5
		2	11	510	2	2.1	3
510	2	1	19	716	2	1.1	4
716	2	1	20			2.2	7
512	3	1	25	512	3	3.3	4
		2	16			3.6.1	5
		3	18			4.2	3
		4	17			1.1	6
514	4	1	10	514	4	1a.3	2
		2	22			3.1	5
		3	31	587	4	1b.1	3
		4	13	593	5	1	6
587	4	2	17			2	4
76	5	4	16	76	5	1	6
		2a	27	647	6	1	3
2003	6	1	23	671	7	1	1
		2	19	1072	8	2	5
				2069	9	1	3
				2079	10	3	4

ATTEMPTS TO INDUCE CHLAMYDOSPORE FORMATION IN VITRO

With a view to avoiding the necessity of inoculating the host plant, an effort was made to induce chlamydospore formation in pure culture. Some investigators have reported chlamydospores by some species of the *Ustilaginales in vitro*. Kniep (18), who obtained chlamydospores of *Urocystis anemones* (Pers.) Kniep non Winter, was the first to obtain the complete life cycle of one of the smuts *in vitro*. Chlamydospore production in one or more of the species of *Ustilago* has been reported by Fleroff (11), Sartoris (23) and Wang (28). Various compounds were added to a modified Czapek's agar and the media were inoculated with compatible lines. A number of concentrations of β -indole-3-acetic acid, indole-3-propionic acid, vitamin B₁, vitamin C, panthothenic acid, and nicotinic acid were used. Extracts of host plants, sterilized by steam under pressure and by filtration, were added to agar. Shredded plant material also was mixed with agar. Compatible lines growing adjacent to each other were irradiated with ultraviolet light, using the 2537 Å line and a dosage of 4000 ergs/mm². None of the methods tried was successful. Failure to obtain typical chlamydospores of *U. zeae* in pure culture by the methods employed in this study does not preclude the possibility of their formation. It is possible that some labile substance is present in the host that induces chlamydospore formation.

INHERITANCE OF FACTORS

a. Growth Type

Sporidial and mycelial types of growth have been described by Kernkamp (17) and by Stakman *et al.* (25). Isolations were made from 4 field collections of galls and in every case the isolates were sporidial in type of growth. The mycelial types studied in this investigation arose as mutations from sporidial-type colonies. They remained mycelial in type through successive subcultures. Examinations of microscopic mounts from colonies of mycelial type revealed a few sporidia among the hyphal segments. Although sporidia predominated in the sporidial-type colonies, a few short hyphae were found. Single hyphal segments isolated from sporidial-type colonies yielded colonies of that type. Likewise, single sporidia from mycelial-type colonies in turn yielded mycelial-type colonies. Crosses between monosporidial lines of sporidial type and mycelial type gave a preponderance of 1:1 segregations, but 3:1 and 1:3 ratios for both types were present. Crosses between mycelial lines always yielded mycelial types and sporidial-line crosses yielded only sporidial types. Nine complete sets of sporidia were isolated from a chlamydospore formed by crossing third-generation mycelial and sporidial types. Each of the sporidia from cells 1 and 2 gave rise to mycelial-type colonies; those from cells 3 and 4 produced sporidial-type colonies.

Sporidial-type colonies frequently formed sectors of mycelial type, but mycelial-type colonies never were observed to form sporidial-type sectors. They did, however, form other mycelial-type sectors.

A comparison of the rate of sectoring of colonies of the 2 types of the 5th inbred generation was made. Of 570 colonies of sporidial type allowed to grow for 5 weeks on corn-meal agar at 25–27° C., 430, or 75.4 per cent, formed sectors. Of 451 colonies of mycelial type, 132, or 29.3 per cent, formed sectors. From these results it appears that the mycelial-type colony is approximately 3 times as stable as the sporidial type.

b. Sex

In the first 3 inbred generations bipolar sexuality existed, but in the 4th generation this was disturbed. Three of the 4 monosporidial lines from one spore were monopathogenic. The monopathogenicity of one of these lines was verified by making 3 single-spore isolations from the culture and through introducing these into plants. These inoculations produced galls, but sporidia isolated from the spores in these galls were not monopathogenic. Out of some 4,000 monosporidial lines only the 3 above-mentioned lines were monopathogenic. Christensen (4) and Eddins (9) found a higher percentage of such lines in their material.

c. Color

An apparently uniform mycelial cream-colored (22) line was obtained in the 5th inbred generation from a cross between two similar mycelial

sectors. The 6th generation appeared uniform and identical with the 5th generation parents. The 7th generation, however, segregated beige and cream color. The sudden appearance of the beige color was perhaps due to the segregation of a modifier for color. Each of these types was established as a uniform line in the 8th generation. The beige-color line was inbred to the tenth generation.

SEGREGATION

Segregation of factors for sex, color, and type of growth occurred in either Division I or in II as determined by seriation of the promycelial cells. For each of these characters 1:1, 3:1, and 1:3 ratios were found and a 4:0 segregation was found in one case for sex. The 3:1 and 1:3 ratios may be due to delayed segregation, as has been shown by Dickinson (6) for *Ustilago avenae* and *U. levis*. To make certain of this at least three successive sets of sporidia should have been isolated from the spores in question.

MUTATION

The sectors and "patch mutants," which appear from time to time on colonies grown upon most laboratory media at ordinary temperatures, are regarded as mutations. Their uniformity in successive subcultures and the fact that they can be recovered intact following crosses indicate that they have a genetic basis. The writer agrees with Stakman *et al.* (25) that the so-called "losses" of virulence often reported in the literature are due to mutation. In the course of this investigation no gradual changes in characteristics were ever observed. The fact that sectors in turn gave rise to other sectors renders delayed segregation unlikely as an explanation for the phenomenon of sectoring.

SUMMARY

Spores germinated in from 12 to 24 hours on Czapek's agar at 25–30° C. At lower temperatures germination was delayed and atypical. Spores subjected to monochromatic ultraviolet irradiation exhibited delayed germination. The lag period increased with increases in dosages.

Chlamydospores exhibited a high percentage of germination immediately after collection, even though the galls were not always entirely dry. No indication of a "rest period" was ever found.

Plants of all ages were found susceptible to *Ustilago zeae* so long as meristematic tissue was present and the temperature was favorable to infection.

At temperatures below 21° C. no infection of the host was obtained with compatible lines in the greenhouse. There was, however, anthocyanin formation. At temperatures above 27° C. high infection was obtained.

The medium was found to exert a pronounced effect upon the appearance of colonies and mutation rate. Cultures on Difco corn-meal agar gave rise to fewer mutations than did those on more nutritive media. Czapek's agar was used to maintain stock cultures.

The measures employed to prevent mutation in stock cultures included

incubating them at temperatures below 18° C. on a medium low in available nutrients with frequent transfers to fresh lots of media.

Mutations were not induced by ultra-violet irradiation, by X-rays, or by brief exposure of sporidia to temperatures near the thermal death point.

Increases of incubation temperature within limits increased the frequency of mutation. Below 20° C. few, if any, mutations were found. Thirty-seven and a half degrees C. was found to be above the maximum for the lines studied.

The frequency of mutation was not affected by inbreeding for ten generations.

A number of unsuccessful attempts to induce chlamydospore formation of *U. zeae* in pure culture were made.

Stocks exhibiting the characteristic mycelial type of growth, sporidial type of growth, and beige and cream color were established. The sporidial and mycelial types of growth were established in the parental generation and inbred as far as the seventh and tenth generations respectively. Beige and cream colored lines were established in the eighth inbred generation and the former was inbred to the tenth generation.

The mutation rate of sporidial lines was approximately three times that of mycelial lines.

Segregation of factors for color, for sex, and for type of growth occurred in both I and II of the reduction divisions.

Three monosporidial lines out of some 4,000 were monopathogenic. Spores from galls produced as a result of inoculation with one of these lines did not give rise to monopathogenic lines.

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ONION EELWORM ROT OR BLOAT CAUSED BY THE STEM OR BULB NEMATODE, *DITYLENCHUS DIPSACI*

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REVIEW OF LITERATURE

"Eel-disease," "Kroefziekte," or onion "bloat," has been known in Europe since its first observance by Kühn (10), whence it may have come to America. Kühn named the causal organism *Tylenchus putrefaciens*. Beijerinck (1) called it *Tylenchus allii*, but Ritzema Bos (16, 17, 18) showed it to be the same species as that described from teasel (*Dipsacus*), narcissus, hyacinth, and rye. He succeeded in transferring the strain from onion to rye and from hyacinth and rye to onion. Ramsbottom (15) and others have transferred the narcissus strain to onion, and Walton (22) reported mass transfers of the onion strain to parsnip. He also found (23) the disease could persist in soil 4 to 5 years.

From the descriptions of the disease given by Chatin (3) in an early thesis in Paris, by Beijerinck (1) and by Ritzema Bos (18), it is known that the nematode may infect the bulb, the stem, leaves, flowers, and occasionally even a small percentage of the seeds, although diseased plants are said to go to seed rarely. Besides onion, Goodey (8) now lists shallot, leek, garlic, chives, and wild onion (*A. vineale*) as susceptible.

HISTORY OF THE DISEASE IN THE UNITED STATES

Although recorded by Bessey (2) as occurring on rye as early as 1907, and by Smith (19) as affecting red clover in the northwestern States 20 years ago, the first authenticated record of *Ditylenchus dipsaci* (Kühn) Filipjev on growing onions in this country according to Steiner (20) was an infestation that broke out in 1929 or 1930 on the muck farm of James Dellaquila near Canastota in Madison County, New York. To be sure, it had been found before that on ships' stores and is listed by McKay (12) not only as a serious pest on strawberry and clover in Oregon but also as occurring on onions somewhere in the United States. Godfrey and Scott (7) found it on garlic in California and recorded that this strain could attack salsify, parsley, and even celery. This brought the number of cultivated food plants known to be susceptible in America well above 30 out of some 195 hosts recently listed by Steiner and Buhner (21).

In view of the apparent virulence of the attack at Canastota, where a one-crop system of muck farming is well entrenched, and of the devastation reported by Cobb (5) as occurring in New South Wales, where a similar situation existed, vigorous steps were taken to stamp out this infestation. This was done in 1931 by steaming about 15,000 square feet of muck with a pair of inverted pans operating on a 30-horse-power traction engine loaned by the Town of Canastota. Treatment was done between August 24 and 29, when the muck was in a favorable condition for quick penetration of the steam. Temperatures above 180° F. were obtained 6 inches below the surface after 20 minutes of steaming. The cost of this treatment, including construction of the two pans, labor, coal and piping (Fig. 1, A and B) was \$230.00, borne by the Bureau of Plant Industry of the New York State Department of Agriculture. The work was supervised and reported by the senior writer (13). Lettuce and celery were grown on this field the following year as further precaution. Numerous subsequent surveys during the next 8 years covering this and adjoining farms revealed no further trace of the disease. While it has not been reported recently on onion in other States, the reason simply may be due to no one having recognized it.

In 1938 this disease was again discovered on 3 farms a few miles distant from the Dellaquila field. The same appearance of poor stand, weak prostrate foliage, suggesting lightning injury (Fig. 2), followed late in the season (August 20) by much rotting and splitting of bulbs (Fig. 3), was in evidence. All growers reported that the trouble had been of several years' duration, worse some seasons than others, and definitely on the increase.

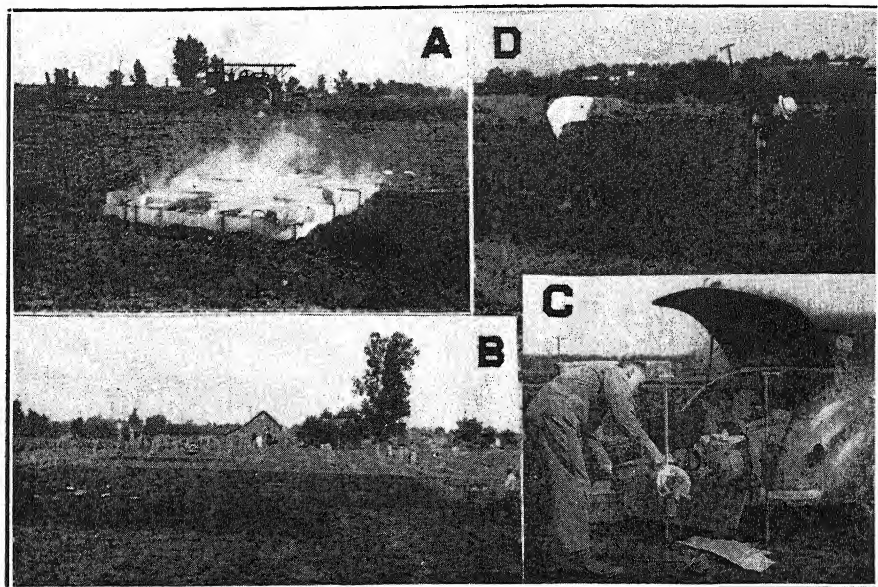


FIG. 1. Field views of eradication methods employed. A. Steaming at Canastota with inverted pan. B. Two infested areas covering nearly 15,000 sq. ft. were steamed in 6 days at a total cost of \$230. C. Three smaller areas have been treated as shown here by injecting chloropierin at close intervals. D. Filling the injectors, showing method of handling chloropierin in the field.

One grower had given up trying to raise onions on about one-fourth of an acre where the trouble was the worst.

Again, the New York State Bureau of Plant Industry under the direction



FIG. 2. Portion of a diseased area of Early Yellow Globe onions on muckland in Madison County, N. Y., taken August 13 (by R. L. Clement) showing thin stand, weak foliage, stunting and many dead outer leaves.

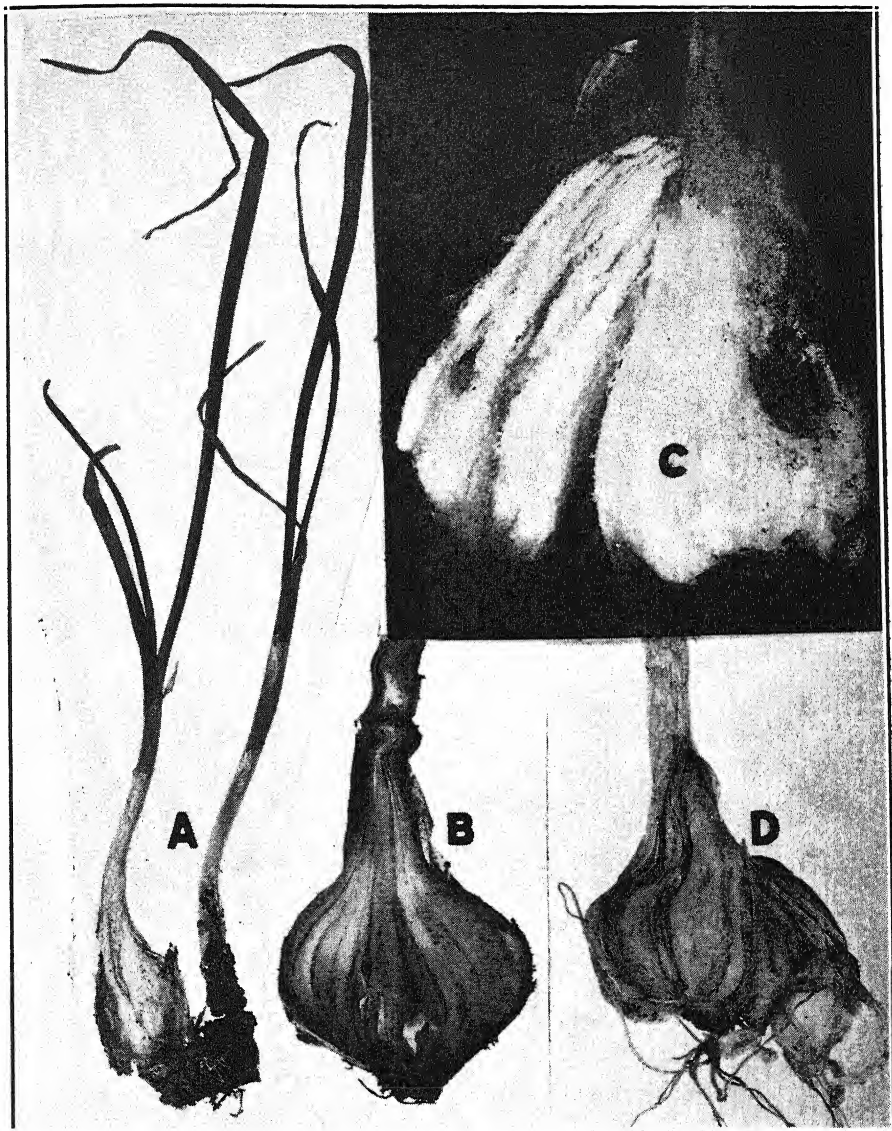


FIG. 3. Bulbs with rot caused by nematodes and associated injuries. A. A plant 14 weeks after inoculating, through tip of one leaf, and showing new top growth after brief dormant period and small, ragged, mealy bulb heavily infested, $\times \frac{1}{2}$. B. A bulb of Early Yellow Globe from field, showing typical *Fusarium* dry-rot symptoms for comparison with C, $\times \frac{1}{2}$. C. A typical *Ditylenchus*-infected, half-grown bulb with outer scale peeled aside to show white, frost-like mealiness. D. A malformed split bulb commonly found in infested areas but not always infected.

of A. B. Buchholz undertook to stamp out the infested areas in these 3 fields. The difficulties incident to steaming seemed insurmountable with the available funds, so sulphur was applied to some of the spots and chloropicrin to one of the largest. The sulphur was broadcast the first week of September

at rates between 1,200 and 3,000 pounds per acre and was then disced in. The chloropierin was applied through the courtesy and by agents of Innis, Speiden and Company, New York, at approximately 350 lb. per A. in rows and holes 15 in. apart, 8 in. deep, at 4 cu. cm. per hole.

Examinations made in July and again in August, 1939, indicated that applications of sulphur at rates less than 2800 lb. per A., while helpful in reducing infections to a point where onions could perhaps be grown without too much loss, were still not adequate to bring about eradication, at least in one year, according to Chitwood *et al.* (4). On the other hand, where the disease seemed to have been wiped out by the heavier sulphur applications, the onions did not attain full market size. This probably was due to unfavorable pH, which was 4.72 outside and 3.78 inside some of the sulphured areas 10 months after application. The chloropierin-treated area grew an excellent crop of onions apparently free from nematode injury, conditions having been very favorable with respect to soil moisture and temperature at the time of application.

A survey reported by Newhall *et al.* (14) covering about 1,000 acres in the Canastota area, made in August, 1939, by the writers with the aid of R. L. Clement and I. D. Smith, inspectors of the New York Bureau of Plant Industry, failed to reveal any other infested fields. Less extensive surveys, including 5 acres in the vicinity of Lansing, 125 acres at S. W. Oswego, 7 acres near Fairhaven, 3 acres near Victory, and 125 acres near Savannah, also uncovered no more infestations. But the junior writer and Mr. Clement each found a field between Pine Island and Florida in Orange County, New York, 140 miles (air line) from Canastota, in each of which approximately 6,000 sq. ft. were heavily infested. There is reason to suspect the origin of one of these areas to be the same as Dellaquila's, *i.e.*, set onions originating outside of the State in 1929.

The possibility of the outbreaks recorded here having come from such wild hosts as dandelion, teasel, or strawberry has been considered. Godfrey (6) reported *Ditylenchus dipsaci* on dandelion in western New York and Ontario, Canada, years before. But none could be found in the vicinity of the fields nor was infection secured on dandelion with mass transfers from onion in one trial by the junior writer.

SYMPTOMATOLOGY

On Seedlings

Onion seed of the variety Ohio Globe was sown in steamed muck to a portion of which was added a suspension of nemas from chopped, infected onions. The soil was kept moist with a mist spray. Emergence of the seedlings was considerably retarded (Fig. 4, C). Stands were reduced from 69 per cent in steamed but noninfested muck down to 47 per cent in the infested muck. Over half of the seedlings that did emerge were diseased. Many of the living seedlings were very pale and assumed thickened, arched, and abnormal shapes by the time they were $\frac{1}{2}$ in. high. Some of these are shown

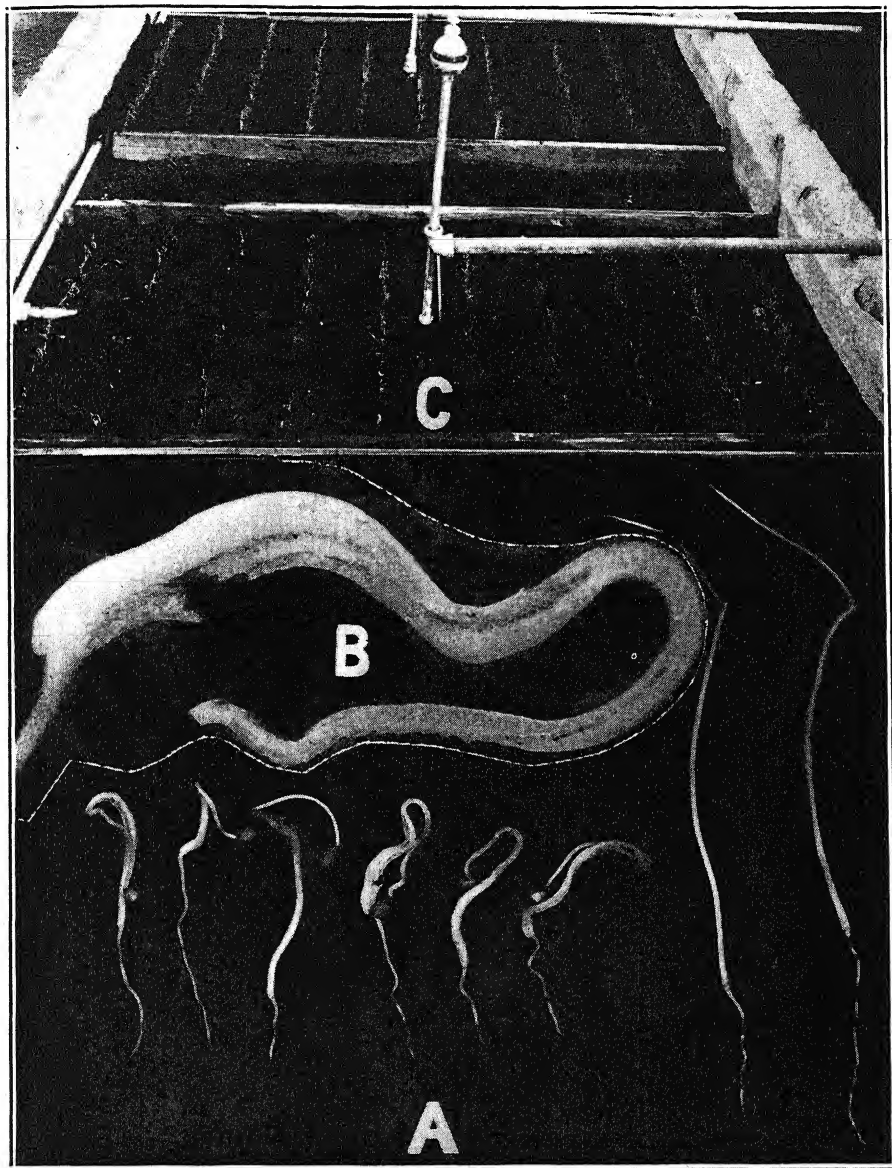


FIG. 4. Seedlings of onions 10 days after sowing in sterilized and reinoculated muck. A. Early symptoms of hypertrophy, distortion, pallor, and cracking on six diseased seedlings compared with 2 normal ones on right, all natural size. B. One of six diseased seedlings, $\times 5$; note puffy, lace-like cracked and twisted condition of entire cotyledon above the stem plate. C. Inoculated bed in foreground where the stand is but 46% of the check and over 50% of the seedlings showing above-ground are diseased. A mist-spray nozzle seen in the center of each bed was used to keep soil moist.

in figure 4, A and B, all in the cotyledon stage. Enlargements somewhat suggestive of those caused by smut, *Urocystis cepulae* Frost, were pronounced, but these are readily distinguished by their color. Splitting of

the epidermis often followed the enlargement. Many seedlings died during the first 3 weeks after sowing, and the survivors sometimes died later. This agrees with growers' observations of the progressive failure of the crop on infested soil. Living nemas could easily be found in the swollen tissues of the seedlings above the stem or root plate but not below it (Fig. 4, B). Over 50 were found in one such seedling 3 weeks after sowing, before the first true leaf emerged.

On Older Plants

When onion sets were planted on artificially infested muck in the greenhouse, the first symptoms were observed after 3 weeks and included stunting, light-yellow spotting of the foliage (decolorization), swellings, and open lesions, all of which come under the general heading of "spikkles" or eel-worm spots, as that term is applied by Dutch bulb growers. At this time nemas could be found in leaves and young bulbs, though the latter showed no visible symptoms.

The first field symptoms the writers have seen on onions grown from sets were observed in July as slight stunting coupled with a flaccid condition of many outer leaves. A necrosis or die-back of outer leaf tips and a general weakened condition of the older leaves that prevented them from growing erect was very noticeable (Fig. 2). Within the leaf tissue of diseased plants and, particularly the outer layers of stem tissue, the nema has been found in abundance under the microscope. "Spikkle" symptoms were not seen in the field in 1938 or 1939. Godfrey and Scott (7) found that, on garlic, these symptoms also are lacking.

As the bulb swells to half its final size in July and August, the nemas seem to migrate down from the leaves to the outer scales of the bulbs, where their activity results in a more or less pronounced softening of the parenchyma. At this time a little oblique pressure with the thumb on the upper half of the bulb may result in loosening the outer scale and revealing the soft mealy tissue beneath (Fig. 3, C). This lace-like mealiness looks like thick frost. It becomes more pronounced in the normal course of events as the season progresses and more nematodes develop in the tissues of the rapidly growing bulb. Perhaps ten times as many bulbs showing these symptoms were found in August as in July in the same field plots.

Under the microscope this mealy character is seen to be due to the disappearance of much of the middle lamella in the parenchyma of the bulb scales, leaving the cells in a loose granular pack. The nematodes wander about mostly between the cells, but they have been found within them.

Secondary Invaders May Alter Typical Symptoms

Not infrequently examination of suspected bulbs and diagnosis of *Ditylenchus dipsaci* infection is rendered difficult by the presence of so many other invaders. The commonest nematodes found have been *Rhabditis* spp., *Aphelenchus avenae* Bastian, species of *Pristionchus*, *Panagrolaimus subelongatus* (Cobb) Thorne, and *Aphelenchoides parietinus* (Bastian) Steiner.

Of these, *Aphelenchus* and *Aphelenchoides* are the most difficult to distinguish from *Ditylenchus*. Mites (*Rhizoglyphus hyacinthi* Boissduval) and larvae of thrips (*Thrips tabaci* Lindeman) and several maggots may also follow so closely on the heels of *Ditylenchus* as to greatly alter the true picture of a white, mealy, frosty or lacy, dry, non-odorous rot. The presence of these secondary organisms, accompanied as they usually are by many bacteria, results in a moist, malodorous condition and renders the bulb a less congenial place for *Ditylenchus*, which is not a scavenger but an active parasite on living tissue.

Infection Experiments

Dry, cured onion bulbs have been artificially infected by the authors with mass transfers of *Ditylenchus dipsaci* from onions and from narcissus. The easiest way to keep this nematode in culture in the laboratory has been by such transfers, the bulb being stored in a cool basement, moisture kept in by wax-paper wrap. After thirty days, the disease may progress to the base of the bulb and occasionally involve every scale.

When a suspension of nemas in a drop of water was placed on 12 sound, uninjured Early Globe bulbs, from which the outer brown scales had been removed, and the onions kept in a moist chamber at room temperature for a month, no signs of infection were observed. But when the skin was previously broken, infection could be detected in two weeks as a soft swelling for half an inch about the point of injury. Laidlow and Price (11) reported that onions transplanted from sterilized to infested soil were not liable to attack unless the bulb is injured in the process or, subsequently, by other agencies.

Red Weathersfield onions growing in the greenhouse were readily infected July 20 by injuring the tips of leaves and injecting a few drops of a water suspension of *Ditylenchus dipsaci*. The resulting bulbs from these plants were small and heavily infected at harvest time 8 weeks later. One plant, which had split to form 3 bulbs, produced 2 normal, healthy ones and 1 rotten bulb of equal size. The nemas had not passed from diseased to healthy bulbs, even though all were attached at the base.

During storage, activity of the nemas continues and bulbs become lighter in weight, somewhat puffy, and more yielding to pressure of the fingers. As deeper layers of the onion become invaded the bulb may swell enough to suggest the term "bloat," which is really only applicable to some bulbs in this storage condition. Secondary rots may obscure this symptom.

DISCUSSION

Control of this disease in a region where onions are grown almost continuously is difficult without equipment for steaming between crops. Such equipment is not always readily available. Chemicals have been resorted to in the past 2 seasons, but it is too early to give a clear and authoritative evaluation of them. As already mentioned, the indications are that on muck underlain with marl, but having a pH close to 5.0, 1.5 tons of sulphur

thoroughly disced into the top 6 inches may eradicate the nema. But such heavy applications are likely to reduce the yield of the first crop of set onions considerably. Applications of less than 1 ton failed to eradicate in 3 field trials. Chloropicrin, in one field trial at 350 pounds per acre, applied just before a heavy shower, seemed to give complete control, with no crop injury, but is expensive for large-scale eradication. Further tests with these materials were made in 1939 in several fields in Madison and Orange counties (Fig. 1, C, D) and will be reported on later.

Three greenhouse attempts to prove the existence of migrant *Ditylenchus dipsaci* in muck from infested areas by growing a crop of several thousand onion seedlings have all failed. In these cases all onion bulbs that might have harbored the nema were screened out. When infected bulbs previously chopped were mixed with the soil, the resulting infections were numerous (Fig. 4). It must be admitted the soil used in these tests had lain in the field during several weeks of unprecedented, dry, warm weather and had become almost powdery-dry. While not denying the possible persistence of nemas living free in the soil, the writers are inclined to stress diseased bulbs as of far greater importance in the overwintering of this nema. Laidlow and Price (11) reported its survival after 2 years in dried onion plants and after 6 months in dry soil.

In view of the fact that the strain of *Ditylenchus dipsaci* attacking onions has been transferred to rye and the one from rye to onions, there is a question about the wisdom of using either this cereal or oats as a cover crop on infested muck. Since the strain attacking hyacinth and narcissus has been transferred to onions, these crops should be kept a safe distance from soil intended for onions, especially since the disease is known to occur rather commonly on these ornamentals.

The safest crops to use in rotation with onions on infested muckland where maximum returns must be secured would seem to include carrots, lettuce, spinach and beets. Unfortunately, celery and potatoes are listed among the susceptible vegetables.

The length of the rotation that may be needed to completely starve out this pathogen from muckland is the subject of experiments now under way, but some indication may be afforded by the work of others concerned with upland infestations. In England, Hodson and Beaumont (9) have obtained evidence that 3 years is long enough, provided no susceptible weeds are present, such as ribwort plantain, but Walton (23) states that it may persist for 4 or 5 years. Godfrey and Scott (7) report a case in California in which 6 years of continuous lettuce culture apparently starved out the strain attacking garlic.

The results from rotation and fallowing probably depend largely upon the soil moisture and flora. With sufficient moisture the nemas are active, seeking new hosts in consequence of which they use up the foods stored within. Goodey (8) reported laboratory experiments indicating the starva-

tion of this species in moist soil after 12 to 18 months, and the junior writer has noted starvation on agar plates after shorter periods.

It is of more than passing interest that the pest is known to occur in California on garlic and has been found to pass over from this crop to salsify, parsley, and even to celery. Godfrey (6) showed that the strain attacking false dandelion (*Hypochaeris radicata*) is carried within the seed, while he also found evidence indicating that this strain was introduced into the country when that weed was probably brought to our shores many years ago in soil used as ballast by lumber boats. Other cases of seed-borne nemas of this species are well-known. In this connection, Ritzema Bos (18) reported obtaining as high as 3 per cent infection of seed from diseased onion plants. It is, therefore, important as a precaution against further dissemination of this disease that both sets and seeds be grown a good, safe distance from any infested fields.

SUMMARY

1. Records of outbreaks of *Ditylenchus dipsaci* occurring on growing onions in North America are confined to New York State, but the likelihood of these all tracing back to commercial sets sold in the State in 1929, but grown elsewhere, indicates that this disease may exist in other places.

2. A description of the disease as it affects onion seedlings and growing bulbs is given. On the former, a stunting, distortion, temporary decolorization and hypertrophy are characteristic. On the latter, a softening of outer scales accompanied by frostlike, odorless, mealiness of the parenchyma tissue is fairly diagnostic.

3. In the absence of rotation, the disease may reduce yields to almost nothing.

4. It has been eradicated once by steam sterilization of one-third of an acre of muckland.

5. The use of sulphur and of chloropicrin as more economical soil treatments have shown some promise in preliminary tests.

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EFFECTS OF SOIL TYPE, SOIL STERILIZATION, AND SOIL REACTION ON BUNT INFECTION AT DIFFERENT INCUBATION TEMPERATURES

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INTRODUCTION

It is known that the degree of infection of a susceptible variety of wheat with *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn is dependent upon a number of environmental factors. Experimental data indicate clearly that soil type (3, 8, 9, 13), soil temperature (3, 5, 7, 8, 9, 10, 14), and soil moisture (2, 3, 7, 8, 9, 14) are all involved and any one of these factors may limit infection. Less is known of the effect of post-infection environmental factors. Faris (3) found no evidence that the growth of the host, after the germination stages, had marked effect on the development of bunt in the winter varieties Dawson and O.A.C. No. 104. Smith (12), however, has shown that high temperature during this period limits the development of smut in Hope wheat but not in Jenkin. Variations in these environmental factors as they occur in different wheat-growing areas, affecting both the host and pathogen, undoubtedly account for differences in the response of wheat varieties to any one race of *T. tritici* or *T. levis* (11). As part of a comprehensive study of

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this problem, tests were made at the Arlington Experiment Farm, Arlington, Virginia, involving the effect of soil type, soil sterilization, soil reaction, seed size, and different temperatures during the seedling growth stage.

METHODS AND EXPERIMENTAL RESULTS

Soil Type

In studies on the effect of soil type as a factor influencing infection of wheat by *Tilletia tritici* and *T. levis*, comparisons for the most part have been made between the effects of light sandy soils, presumably low in organic matter, and heavier soils higher in organic content. Results obtained by Faris (3), Volk (13), Rabien (9), Leukel (8), and others indicate that the latter types are more favorable for bunt infection than are the former. In the present experiment, two productive soils were used. One obtained from University Farm, St. Paul, Minnesota, was mapped in the Ramsey County, Minnesota, soil survey (1914) as Hempstead silt loam. It was a surface soil of a dark silt loam high in organic matter with a pH value of 6.7. The second type was the surface soil of a Mendon loam obtained from the Experiment Station Farm at Logan, Utah. This was a grayish-brown, friable silt loam, free from harmful accumulation of salts or alkali, containing little or no free lime and with a pH value of 8.1. These soils were adjusted to approximately 50 per cent of their moisture-holding capacity with sterile distilled water and placed in galvanized-iron germination pans, 2 inches deep, 4 wide and 8 long. Seed lots of Marquis (C.I.² 3641) and Thatcher (C.I. 10003) wheat were each separated into large and small seed by using a No. 8 Tyler screen (8 meshes to the inch). The kernels that did not pass through this screen were considered large and those that did were considered small. These lots of large and small kernels were inoculated with chlamydospores of *T. levis*, race L-2 (11), at the rate of 0.5 grams of chlamydospores to 100 grams of seed. Three replications of 200 seeds each were planted at 1 inch depth in the soils, placed in incubation chambers at 5°, 10° and 15° C., and left there until the seedlings emerged from the soil to a height of approximately 1 inch. The seedlings were then transplanted to greenhouse beds, where all were subjected to the same post-infection environmental conditions until maturity. Thus, under the conditions of these experiments, differences in percentages of smut at maturity should be due to environmental influences effective only during the incubation periods. The resulting data are given in table 1, and those giving the responses of the Marquis wheat grown from large seed are shown graphically in figure 1.

From the above data it is clear, as pointed out by previous workers, that soil type may influence the degree of infection of wheat with *Tilletia levis*. Apparently the differences in effect that have been observed between the light sandy-type soils and those higher in organic matter hold also for 2 productive soils used in these tests, both of which are high in organic matter.³ The

² C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

³ Organic matter expressed in percentage total nitrogen was found to be 0.264 for

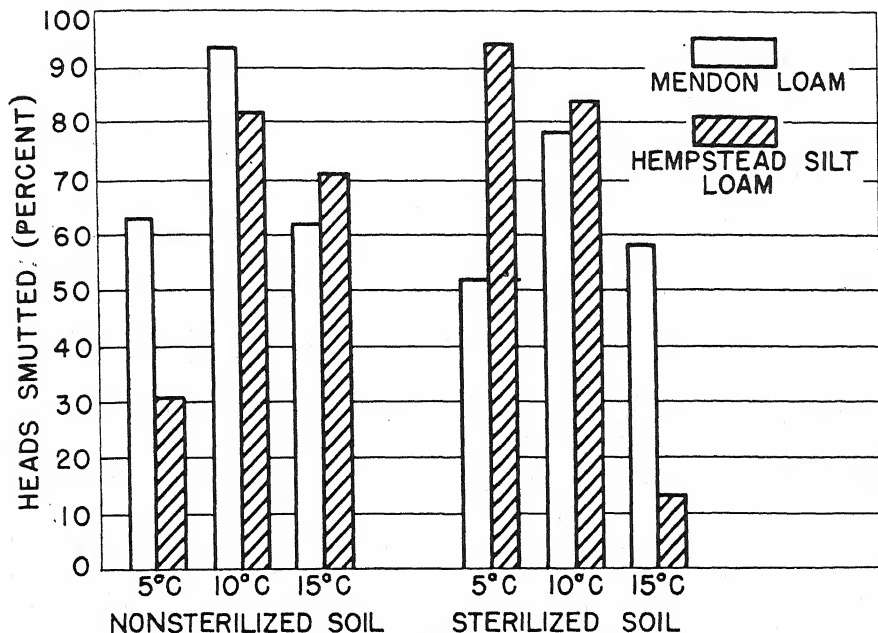


Fig. 1. Percentages of infection in Marquis wheat, large seed, germinated in non-sterilized and sterilized Mendon and Hempstead silt loams.

present data indicate, however, that the differences in effect in the 2 soils are contingent upon the temperatures prevailing during the incubation period. At incubation temperatures of 10° and 15° C., there were no marked differences in the effect of the 2 nonsterilized soils on the degree of infection of Marquis wheat. At 5° C., however, there were marked differences. When the inoculated large seed was germinated in the Mendon loam there was 63.9 per cent smut as compared with only 30.9 per cent in the Hempstead silt loam. In comparable tests with Thatcher, higher percentages of smut developed where the inoculated large seed was germinated in the former than in the latter soil at all 3 incubation temperatures. However, as with Marquis, the greatest differences were at 5° C., 72.5 per cent developing in the Mendon loam as compared with 30.3 per cent in the Hempstead silt loam.

In general, less vigorous wheat seedlings develop from small seed than from large. Since, in some years, only small and shrunken seed of a variety is available, size of seed was made another variable in these experiments. Heald (4) and Holton and Heald (6) have found that percentages of bunt infection may vary, depending upon the origin of the seed. In order not to introduce this variable, the lots of large and small seed used were of the same origin.

Bayles (1) found a tendency for the seedlings from smaller seed of Marquis to be more susceptible to bunt than those from the large. In the

the Mendon loam and 0.196 for the Hempstead silt loam. The writers wish to express their appreciation to Mr. P. R. Dawson of the Division of Soil Fertility, B.P.I., for these analytical data.

results obtained by the writers with Marquis and Thatcher differences in percentages of infection in the large and small seed were negligible with an incubation temperature of 10° C. On the other hand, at 5° C. and 15° C., there were slight, though consistently higher, percentages of bunt in the plants from the small seed. The results in this experiment also emphasize the fact that any differences in the effect of the two soils were contingent upon temperatures prevailing during the incubation period.

Soil Sterilization

In certain wheat-growing areas, infection from soil-borne chlamydospores of *Tilletia* spp. is common. It is, therefore, necessary to sterilize such soils for use in controlled, greenhouse experiments involving pathogenicity tests with individual races. The question arises whether pathogenicity tests in sterilized soils are comparable with those made in nonsterilized soils. The sterilization was accomplished by subjecting portions of each soil before planting to steam at 15 lb. pressure for 4 hours on one day and 2 hours on the next.

Data on the response of the two varieties (Marquis and Thatcher) to *Tilletia levis*, race L-2, when incubated in the sterilized and nonsterilized soils, are recorded in table 1, and the data on the response of Marquis are shown graphically in figure 1.

With Mendon loam, steam sterilization effected a reduction in percentages of infection in Marquis and to a somewhat more marked degree in Thatcher at each of the 3 incubation temperatures. With the Hempstead silt loam entirely different results were obtained. In this soil, steam sterilization effected marked increases in infection in both varieties when incubation temperatures were at 5° C., little or no effect at 10° C., and very marked reduction at 15° C. Thus, for example, for Marquis wheat grown from large seed, the percentages of infection at 5° C. was only 30.9 per cent infection in the nonsterilized soil as compared with 94.6 in the sterilized soil. At 15° C., the reverse effect was obtained with 71.7 per cent infection in the nonsterilized soil and only 13.7 in the sterilized. It is also significant that in the sterilized Hempstead loam the optimum temperature for infection was shifted from 10° C. to 5° C.

A comparison in infection response also may be made between the Mendon and Hempstead soils when nonsterilized and sterilized. When the two soils were not sterilized, the differences in effect on bunt infection were pronounced only at 5° C., incubation temperature. At 5° C., higher percentages of infection developed in the Mendon and Hempstead soils. However, when the soils were sterilized, the comparative effect of soil type is entirely different at both 5° and 15° C. At the former incubation temperature, higher percentages of bunt developed in the Hempstead than in the Mendon soil. On the other hand, at 15° C., the reverse was true with higher percentages of infection in the Mendon than in the Hempstead. Unfortunately, there was not sufficient soil in the original shipment to repeat these experiments in a second season. However, the similarity of results obtained with the two

varieties and with large and small seed of both varieties gives emphasis to the conclusions that are drawn from these tests made in a single year.

Soil Reaction

There are wide differences in the pH values of soils in the same and different sections of the country where bunt tests are being made and the question arises as to the extent to which soil reaction may affect infection by the bunt organisms. Available data are somewhat confusing. Rabien (9) reported that pH 5.0 represents the acid limit for germination in soil of chlamydospores of *Tilletia tritici*. Leukel (8) obtained only 5.8 per cent smut in Purplestraw in soil with a reaction pH 5.6. However, there is evidence that in certain soil types approximately as high percentages of infection may be obtained under conditions of high acidity as of low. The writers obtained 73.5 per cent infection in Marquis wheat grown in an eroded Chester loam from Fairfax County, Virginia, with pH 4.8. Furthermore, when the pathogenicity of 9 physiologic races of *T. levis* was tested on the spring wheat variety Ulka (C.I. 11478) at the Arlington Farm, and at Logan, Utah, in soil of pH values of 5.4 and 8.1, respectively, the lowest percentages of infection obtained with any of the 9 races were 85.5 at the former station and 91.9 at the latter. These differences are considered nonsignificant.

Because of these apparent inconsistencies, tests were made under controlled greenhouse conditions at Arlington Farm with different soil types and different incubation temperatures. Two soils were used. One, obtained near Annandale, Va., was mapped in the Fairfax County, Va., soil survey (1915) as Chester loam. The sample was an eroded type with a pH of 4.8. The second was a surface soil obtained from the Arlington Experiment Farm. It had a pH of 4.8 and was mapped in the same soil survey as Keyport silt loam. To each of these, different amounts of calcium carbonate were added to obtain a range of pH up to 8.1, as indicated in figure 2. All soil reactions were determined by means of a Beckman pH meter. After the addition of calcium carbonate, the soils were adjusted with tap water to 50 per cent of their moisture-holding capacity. Inoculated seed of Marquis wheat was sown in the soil pans, as previously described, and incubated at temperatures of 5°, 10°, and 15° C. There were 4 replications for each soil and temperature condition. When the seedlings emerged approximately an inch, 45 were selected at random from each pan and transplanted to greenhouse beds, the soil of which had a pH value of 5.8. Percentages of infection were obtained on the basis of culm counts. However, under the conditions of these experiments, there was little or no tillering; so, percentages of infection approximate the plant counts. The data are recorded graphically in figure 2.

In both Chester and Keyport soils the percentages of smut increased as the soil acidity decreased over the approximate pH range of 4.8 to 7.0. In no case did an increase in the pH value above 7.39 produce any further increase in smut. The greatest increase in smut with decreasing soil acidity occurred in the eroded Chester loam between pH 4.8 and pH 5.29. These increases

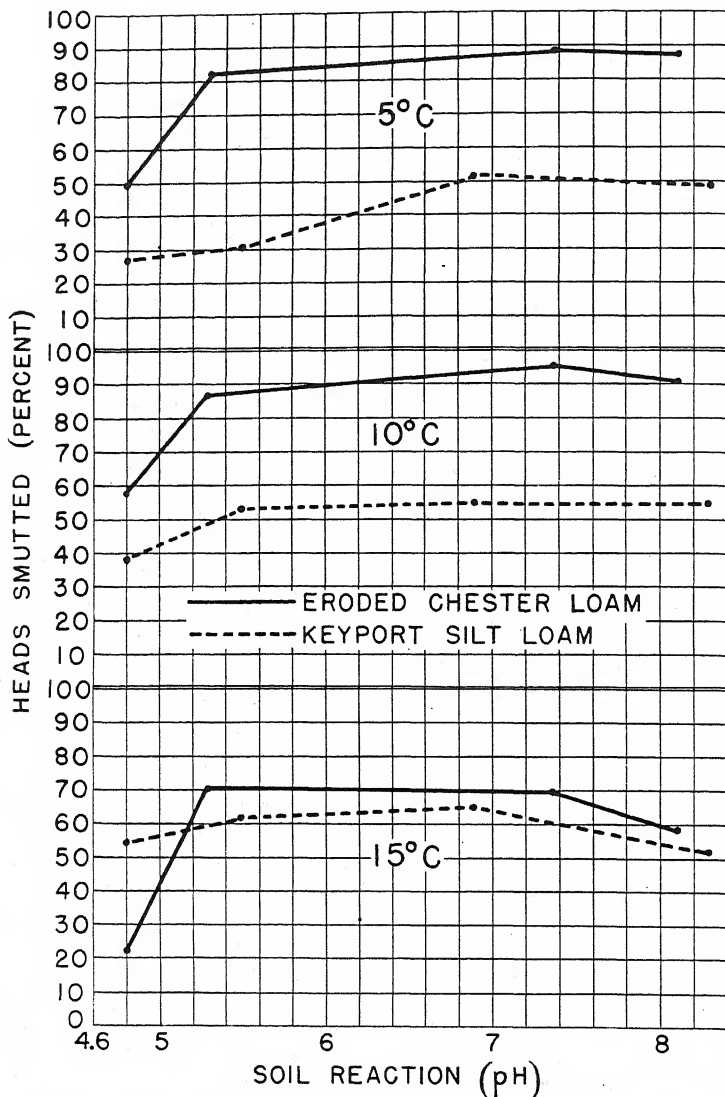


FIG. 2. Effect of soil reaction on bunt infection in Marquis wheat.

occurred at all 3 incubation temperatures. At certain incubation temperatures, however, soil type is a more important factor than the pH value. Uniformly higher percentages of bunt were obtained in the plants from seedlings grown in the eroded Chester loam than in the Keyport silt loam at each reaction tested when the incubation temperatures were 5° and 10° C. At 5° C., the average bunt infection for all pH values in the Chester loam was 76.7 as compared to 39.3 in the Keyport loam. At 10° C., the corresponding percentages were 82.6 and 50.5. When incubation temperatures were maintained at 15° C., differences in the two soils were minimized at all pH values

except 4.8. It will be noted that at pH 4.8 there was 34.7 per cent more smut in the Keyport loam than in the Chester loam, which results are opposite from those obtained at the 5° and 10° C. incubation temperatures.

SUMMARY AND CONCLUSIONS

It is apparent from results obtained that bunt infection in a susceptible wheat variety is affected by soil type. Furthermore, the degree to which it is affected depends on the soil temperature during the period in which infection may take place. For example, when both Marquis and Thatcher seedlings from large seeds were incubated at 10° and 15° C., there were no appreciable differences in the amount of infection in the Hempstead and Mendon loams. At 5° C., however, Marquis developed 33 and Thatcher 42.2 per cent more smut in Mendon than in the Hempstead loam. Similar conclusions may be drawn from an experiment in which a comparison was made between the effect of the eroded Chester loam and Keyport clay loam. At 5° and 10° incubation temperatures, consistently higher percentages of bunt developed in the former than in the latter soil type. At 15°, however, these differences were less, except when the soils were highly acid; then, 34.7 per cent more smut developed in the Keyport than in the Chester loam. Thus, soil type and temperature during the incubation period are closely interrelated in affecting the degree to which a variety may be infected. It has not been determined whether the results obtained are due to a modification of the resistance of the host plant or to the direct effect on the pathogen.

Generalizations may not be made as regards the effect of soil sterilization on bunt infection. Sterilization of the Mendon loam effected reductions in bunt infection in Marquis and Thatcher grown from both large and small seed at all 3 incubation temperatures. Reductions were, in general, greater in Thatcher than in Marquis. With Hempstead loam, on the other hand, the effects of soil sterilization were irregular. At the 5° incubation temperature, instead of a reduction, as occurred in the Mendon soil, sterilization of the Hempstead soil effected an increase in infection from 30.9 per cent in the nonsterilized to 94.6 in the sterilized soil. On the other hand, at 15° incubation temperature, sterilization caused a reduction in infection from 71.7 to 13.7 per cent. The effect of soil sterilization was, in general, less pronounced at the 10° incubation temperature. It may be concluded that pathogenicity tests made in steam-sterilized soils of the Hempstead and Mendon types are not comparable with those made in either of the two soils when not sterilized.

The present experiments on the effect of soil reaction on bunt infection are not extensive enough to answer all of the questions of inconsistency in previous experiments. The data indicate, however, that change in pH may effect a change in the degree of bunt infection in Marquis wheat when germinated and incubated in either the Chester loam or the Keyport clay loam. In both types of soil with an initial pH of 4.8 there was, in general an increase in percentages of infection from the points of high acidity to

a point approaching neutrality, the most marked effect being in the change from pH 4.8 to approximately 5.5. Evidently the degree to which pH affects bunt infection varies with the soil type and incubation temperatures. For example, at 5° and 10° temperatures and at each reaction tested, higher percentages of bunt were obtained in the Chester loam than in the Keyport clay loam; and it is apparent that at these temperatures, soil type affects bunt infection to a greater degree than does change in pH. However, at the 15° incubation, differences due to soil type are less and the soil reaction becomes the more important factor, for at pH 4.8, 34.7 per cent more smut developed in the Keyport than in the Chester loam.

In these experiments there was a trend toward higher percentages of bunt infection when small seed was used in comparison with the large. The seed-size factor may, therefore, be considered a minor factor in effecting variability in response of a variety to bunt. Soil type and certain incubation temperatures were major factors, and the present experiments emphasize the importance of the interrelation of the two factors. Variation in their interrelation may account for seasonal differences in response of a variety to a race of the smut fungus and for inconsistencies in the reaction of a variety to a race of bunt when tested at different places.

It should be noted that in these experiments the effect of the environmental factors referred to has been studied with relation to a single race of *Tilletia levis*, namely, L-2.

BUREAU OF PLANT INDUSTRY,

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ANTHRACNOSE AND CLADOSPORIUM STEM SPOT OF PEONY

FREEMAN WEISS

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The identity of the peony stem spot, described as anthracnose by Whetzel (14) in 1915, has long remained obscure, and specimens have been rarely collected or reported. In routine examinations of peony specimens through a period of over 10 years, the writer has encountered what seems to be this disease but once, on material collected by J. B. Demaree at Willard, North Carolina, August 30, 1938. Anthracnose is not included among the peony diseases observed in Quebec by Coulson (1), nor is it mentioned in the compendiums of diseases of ornamental plants published by Tilford (12) for Ohio, and by White (15) and Pirone (9) for New Jersey. No reference to it is found in the books on diseases and pests of ornamental plants in Europe by Flachs (3) and Pape (8). Although Martin (5) suggested that a red spotting or generalized stippling of stems, leaves, and flowers of *Paeonia*, from which he isolated *Cladosporium paeoniae* together with a budding fungus, was identical with Whetzel's peony anthracnose, a comparison of the descriptions and illustrations of the two diseases shows rather marked differences. The writer (13) suggested the name "measles" for the peony disease, characterized by numerous small red or purple spots on all aerial parts, to which Martin referred. Gregory and Davis (4) described a leaf and stem spot of unknown cause affecting peonies in Indiana, which appears to be of the same type, although one cannot be certain from their illustration.

The North Carolina specimen of anthracnose (consisting of stems only) bore immature acervuli or pycnidia beneath the epidermis in the ash-gray, depressed center of the lesions, in which small numbers of sub-hyaline or slightly greenish, nonseptate spores of elliptical form, measuring $4-6 \times 2-2.5 \mu$, were borne. Other parts of the stem, where the entire epidermis had turned gray, bore dark-brown extruded masses of similar spores. The fungus was provisionally designated as a *Leptothyrium*, but these specimens were inadequate for precise identification or for isolation of the pathogen. However, the correspondence with Whetzel's description and figures was very close.

SYMPTOMS

Red spot or "measles"

Although anthracnose has been rarely observed, the red stem spot, or "measles" type of infection has occurred widely though somewhat infrequently. Strikingly conspicuous examples of this malady were received by the writer from a commercial peony grower of Onarga, Illinois, on June 1, 1924. Martin referred to its prevalence (in the vicinity of Washington, D. C.) in 1929. It occurred here commonly again in 1932. J. R. Kienholz reported this disease to the writer from Oregon in 1932, and A. L. Pierstorff sent specimens from Ohio in 1934. In each instance the plants were promi-

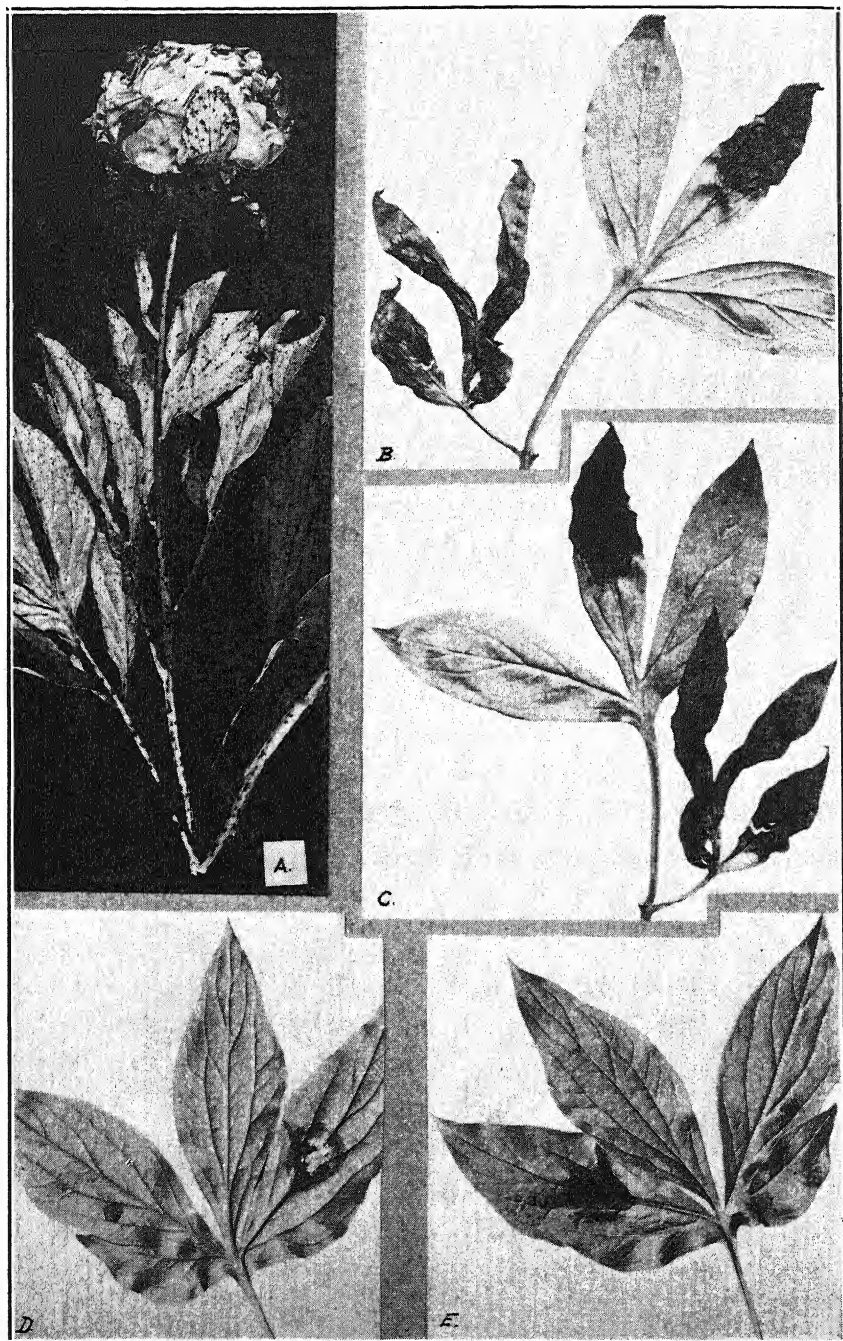


FIG. 1. Spotting of stems, leaves, and flower of peony caused by *Cladosporium paeoniae*. B-C. Lesions on peony leaves resulting from artificial inoculation with a budding fungus isolated from stem and leaf spots; the distinct center and zonate margin are characteristic; B, lower surface; C, upper. D-E. Lesions on peony leaves resulting from artificial inoculation with the conidial stage of *Pezizella lythri*; D, lower surface, E, upper.

nently disfigured by or before the period of full bloom. In June, 1938, a commercial grower of peonies at Concordville, Pennsylvania, submitted specimens of the variety Avalanche from a field which had been severely damaged for flower cutting by this disease.

Since the red stem spot appeared to be increasing in importance a study of it was begun to determine its cause and to clarify its relation, if any, to anthracnose. The discontinuance of this study before its completion makes advisable the publication of the results to date.

Stems, leaf stalks, and blades, floral bracts and petals are susceptible. Sometimes there appears to be a gradient of infection diminishing from near the ground level to the apical parts, or the upper leaves and flower stalk also may be densely spotted. The stem spots are definitely raised, elliptical to elongate, usually discrete but sometimes confluent into streaks, typically about 2-4 mm. long and 1-2 mm. wide. They are very similar to the stem lesions caused by *Cladosporium paeoniae* Pass. as described by Meuli (6). The leaf spots are much smaller, some being mere flecks, less than 1 mm. wide. They are most prominent on the dorsal surface, but the larger spots are visible on the upper surface also. There is a predilection for the veinal tissue, and the spots on the principal veins are larger and more elongate than those on the interveinal portions. The calyx-like floral bracts are attacked similarly to the leaves, and the petals also may bear, sometimes very numerous, small reddish spots of the same pattern. The stem spots increase in size but little after they first become visible, although in mature stems there is a slow extension with loss of the definite margin. Even on mature stems they are essentially superficial, sometimes only one or two cell layers under the epidermis showing disorganization and never the wood. Both leaf and stem spots are typically a purplish or brownish red throughout, usually without differentiation into a lighter center and darker margin; although in age the center may become brown, black, or gray and is cracked and depressed. The reddish color persists in the infected area, even in stems that have died and turned brown. Ordinarily the infected stems, even when heavily spotted, remain alive until the natural end of the growing period.

Sections of the lesions made at an early stage of development show a rather sparse, predominantly intercellular mycelium present in small groups of epidermal and hypodermal cells. The smallest lesion may involve no more than six to ten cells. The contents of the invaded cells coagulate to a brown gummy mass, sometimes with deposition of a purple pigment, and the walls become thin, eroded, and incoherent. There is no association between the lesions and the few stomata present on stems; the leaf stomata are hypophyllous and the lesions may or may not surround them; but, since many of the leaf lesions originate on the veins, infection is clearly not dependent on stomatal penetration. In some sections the internal mycelium bore apically enlarged branches suggestive of the chlamydospores or hyphal knots, and also of the aerial conidia, produced by *Cladosporium* in culture.

The intercellular hyphae were conspicuously intertwined. The surface of old lesions also may bear sparsely the characteristic mycelium and spores of *Cladosporium*. Some of the stem lesions at an older stage become definitely rounded and protuberant, as if in response to the formation of a fungus fruit body within; but, aside from the superficial growth of *Cladosporium*, and sometimes of *Alternaria*, they do not develop definite fruit bodies before winter.

Etiology

Examination of the infected planting at Concordville, in July, showed that not only the red spot was prevalent on stems and leaves but that there was a severe infection of leaf blotch (*Cladosporium paeoniae*), and also a small circular leaf spot having a light-brown center and a dark-brown, or purple, distinct margin. Some of the stems had turned a dark-brown from the ground line to the first or second leaf, but the red-spot lesions could still be distinguished in the general cortical necrosis. Salmon-pink sporodochia were found on some of the dead stems.

Isolations made at this time yielded *Cladosporium paeoniae*, *C. herbarum*, *Alternaria*, and a *Gloeosporium* that appeared identical with a conidial culture of *Glomerella cingulata* Stonem., obtained from *Rubus*. Stems showing different stages of the disease were collected in July and October for storage under various conditions. Arrangements were made with the grower to carry out experiments on the importance of infected stems as a source of the disease in a hitherto uninfested part of the field, and on the effect of removal of stems at various times, from fall to late winter, on the recurrence of the disease in the infested part of the planting.

Examination of the stored stems kept outdoors at this Station, or in a 10° C. chamber, showed in late winter the development of brown pycnidia bearing cylindrical or slightly curved, continuous, hyaline spores. Pure cultures were readily obtained, and the fungus was identified by J. A. Stevenson and Edith Cash as *Pezizella lythri* (Desm.) Shear and Dodge, the pycnidial stage of which is *Sclerotiopsis concava* (Desm.) Shear and Dodge. These colleagues also pointed out that *S. testudinea* Dearness, originally described (2) on dead stems of peony, might be the same fungus, as both concave and convex pycnidia occurred on these stems; the latter is therefore probably to be regarded as a synonym of *S. concava*. It might be noted that further nomenclatorial changes applicable to this fungus have been proposed (7), viz., *Discohainesia oenotherae* (Cke. and Ell.) Nannf. for the ascigerous stage, and *Hainesia lythri* (Desm.) v. Höhn. and *Pilidium concavum* (Desm.) v. Höhn. for the two conidial stages, but their discussion need not be entered into here.

The same fungus also was found on dead peony stems in the Station plot, and on stems from the field at Concordville, which were collected periodically during the spring. Moreover this fungus was isolated by tissue cultures from the first lesions of red spot that appeared on stems of the current year's growth at Concordville, on May 1, 1939. Several collections of *Sclerotiopsis*

on dead stems of peonies imported from Japan have been made by the United States plant quarantine stations at Seattle and San Francisco.

Tests of *Sclerotiopsis concava* for pathogenicity to peony showed that it is definitely parasitic. However, the characteristic lesions of red spot, especially on stems, were not reproduced by artificial inoculation. The degree of succulence appears to be an important factor in its pathogenicity. Inoculation, without wounding, of the youngest available but still fairly mature stems gave negative results. On petioles and blades of young leaves inoculated with a spore suspension without wounding, a diffuse necrosis developed, causing, under moist-chamber conditions, collapse of the stem and the production of large, light-brown, zonate spots on the leaves. Sporodochia corresponding to *Hainesia lythri* were produced copiously on the leaf spots. The fungus was readily reisolated from tissue plantings and from spores. Subsequent tests on mature peony leaves showed but slight pathogenicity, small (1-2 mm.) brown, somewhat angular spots being produced without the development of fruiting bodies. Comparison of these lesions with those previously mentioned as associated with *Cladosporium* leaf spot in the field, suggests that *Sclerotiopsis* probably occurs as a natural parasite on peony leaves. A collection of peony leaf spot submitted to the writer from Suitland, Maryland, in 1934 appears to be of this type but lacks definite fruiting bodies of the *Hainesia* or *Sclerotiopsis* types; it differs distinctly from *Botrytis* or *Cladosporium* leaf spots. The lesions produced by *Sclerotiopsis* on peony foliage are of a wood-brown to fuscous color on both surfaces as contrasted with the chestnut-brown below and taupe-brown to blackish-brown above of *Cladosporium* spot, and are further distinguished from *Cladosporium* spots by the production, at maturity, of the conidial stage *Hainesia*, or the pycnidial stage *Sclerotiopsis*. Although only a few inoculations at controlled temperatures were made, infections occurred only in the range 14° to 22° C., and the lesions developed most rapidly at 18° to 22° C.

The occurrence of this fungus on some 50 different hosts has been reported by Shear and Dodge (11), who also showed by experimental inoculations that it is parasitic on leaves and canes of various *Rubus* spp., on other woody plants, as *Rhus*, *Prunus* and *Salix*, and on several herbaceous plants, especially of the Onagraceae. Its association with weeds, therefore, becomes a matter of importance in its control in peony plantings.

Besides pycnidia of *Sclerotiopsis*, the overwintered stems bore very numerous minute black, superficial or subcuticular sclerotium-like bodies of a different fungus, associated with which there were many rod-shape, hyaline, nonseptate spores averaging $4-6 \times 1-1.5 \mu$. There was also a thin dispersion of mycelium and conidia of *Cladosporium*, cultures of which resembled *C. herbarum* more than *C. paeoniae*.

When stems bearing only the pycnidia of *Sclerotiopsis* were placed among the developing shoots of a potted peony and were thoroughly syringed with water several times, the new shoots developed in 10 to 12 days large,

brown, sunken lesions. When stems bearing the small black sclerotia, together with *Cladosporium*, were similarly used as inoculum, the stems and leaves of the inoculated plant developed in about a week an extremely dense infection of typical red spot or "measles." Reisolations from these spots, on stems or leaves, yielded both *Cladosporium* and a hyphomycete that produced (on corn-meal agar) a submerged or appressed mycelium bearing numerous globular masses of spores pleurogenously or on short lateral branches that were sometimes ramose with slightly inflated sterigmata, but in the main unbranched and bearing terminally a loose cluster of spores. The latter were similar in form and size to those associated with the sclerotia on overwintered stems, though somewhat more variable, some being rod-shape and about $3 \times 1.5 \mu$, others were elliptical in section and ranged from 4×2 to $7 \times 2 \mu$. This fungus also was obtained repeatedly from tissue plantings of natural red-spot infections, usually in association with *Cladosporium*. It is doubtless the same fungus in the "budding phase" that Martin (5) isolated from red-spot infected peonies. Martin also refers to a "fumagoid phase" and states that *Cladosporium peoniae* developed in cultures of the budding fungus. In the writer's cultures also, a fumagoid phase developed with age. Thick-walled, olivaceous to fuscous cells of globose to ovoid form, 8 to 12μ in diameter, developed in simple or sparingly branched chains, or sometimes in irregular aggregates. The different types of aggregates consisted of about 5 up to 50 cells; the larger ones were sclerotoid and were barely visible under a hand lens. In old cultures they imparted a fuscous to black coloration to the entire stroma. No further development was observed in these cultures.

Although the "budding fungus" was often associated with *Cladosporium paeoniae* in isolation plates, it was readily separated by dilution-plating and it, together with its fumagoid phase, appears to be a distinct entity rather than a developmental phase of *Cladosporium*. In the initial stages of its development from a germinating spore in water or on agar, the fungus resembles *Cephalosporium*, but the appressed and viscid character of the mature thallus differs widely from typical *Cephalosporium* species. The fumagoid phase also serves to distinguish it. The form designations *Pseudo-saccharomyces* and *Pseudofumago*, as used by Martin, will serve adequately to characterize it until more information about its life cycle is available.

This fungus also is pathogenic to the peony, causing an extensive, dark-brown, moist necrosis when inoculated into wounded stems, and light-brown, dry spots on leaves with or without wounding. The leaf spots resemble those resulting from inoculation with *Pezizella lythri*, except that no pycnidia or sporodochia are produced, but mycelium and spores typical of the budding phase in agar cultures develop copiously on the surface. Experimental inoculations were successful within the range 10° to 27.5° C., but the growth of the lesions was most active at 18° to 22° C.

Reference has been made to the frequent association of one or more types of *Cladosporium* with peony stem spots. Meuli (6) showed that *C. paeoniae*

caused stem lesions of the red spot type, as well as the characteristic dendri-form leaf spots. In about 200 isolations made by the writer from red spot on stems and leaves, using a 10 per cent Chlorox (5.25 per cent sodium hypochlorite) wash and planting the tissue pieces, without rinsing, on cornmeal agar, *C. paeoniae* was obtained alone in the majority of instances, or associated with the budding fungus. Similar results have been reported by others. In view of the infrequent association of other organisms with *Cladosporium* in lesions of this type, and their inability, even when pathogenic to peonies, to reproduce the characteristic spotting, there is strong circumstantial evidence for regarding *C. paeoniae* as the sole pathogen. The principal questions remaining are what innate or environmental factors determine the small, discrete type of stem and leaf infection as contrasted with the characteristic large leaf blotches and what factors bring about the early-season, pathogenic activity of an organism that has been regarded as able to invade chiefly mature and moribund tissues.

The answer to the first question may be the relatively low virulence of *Cladosporium paeoniae*; that is, the lesions are small and circumscribed when the host tissues are young and vital, whereas infections that are initiated in mature tissues show the characteristic rapid enlargement. Some of the early static lesions also become active as the tissues mature. The relative virulence of the pathogen in this case bears an almost inverse relation to its importance as a disease-producing organism, as it is the profusion of small spots on the stems, foliage, and flowers of the peony during the period of its ornamental value that is economically important. The mature leaf-spot phase is usually of little consequence, except as it creates a reservoir of contamination, as there is ordinarily no serious contraction of the vegetative period or reduction in vigor of the host as a result of leaf blotch.

An answer to the second question was sought in the weather records for seasons of exceptional prevalence of the stem-spot phase, and in the influence of temperature on infection by *Cladosporium paeoniae*. Because of the fragmentary data available on the prevalence of stem spot in different localities and years, no well-marked correlations with weather conditions were expected. In the vicinity of Washington, D. C., the spring of 1929 was outstanding in the last 15 years for the prevalence of this disease. Peony shoot growth is most active here during April and early May. The mean temperature for April, 1929 (57.6° F.), was the highest, and the precipitation in quantity and frequency was the greatest, except for one year, in this 15 year period. In the vicinity of Vincennes, Indiana, an important area of commercial peony culture, there was an outstanding occurrence of stem spot in 1932. The mean temperature there for April (57.6° F.) and May (67.0°) was above normal, i.e., there was an "early spring," but the weather was not exceptionally wet. The outbreak of stem spot at Concordville, Pennsylvania, occurring in 1938, coincided with the warmest April in the last 15 years (53.7°) but precipitation was deficient. Doubtless many other factors influence the development of this disease, but temperature

appears to be one of the most important. An experienced peony grower writes, "The worst infection I have seen was of flowers from the South, and in trips to the South, I see it nearly everywhere there are peonies. No exhibit at the National Peony Show in Lansing, Mich., in 1938, was entirely free from it. I have never seen more than a slight infection anywhere north of Central Pennsylvania."

Experimental inoculations with *Cladosporium paconiae* were successful within the temperature range 10° to 27.5° C., and the lesions were similar in appearance and size from 14° to 22°. At 10° there was a definite increase in the latent period of infection and a decline in the growth rate of lesions. The thermal range of pathogenicity of *C. paconiae*, therefore, includes the temperatures prevailing during the period of peony-shoot growth in spring, and there is a critical point (near 14° C. or 57° F.) where the growth rate distinctly rises.

Control

Field experiments by a peony grower at Concordville, Pennsylvania, and pot experiments by the writer, showed that the infected shoots of the preceding year were the main source of contamination, the soil being secondary. Field plants, which were cut back to the ground in September and October, 1938, were relatively free from stem spot in 1939, and in pot experiments they were quite as healthy as plants produced from roots washed free of soil. Plants left over winter with the tops in place developed stem spot in the spring, both in the field and in pots. Placing infected stems in a previously healthy planting in early March resulted in the appearance of stem spot on surrounding plants in May, with severe infection in a radius of about 2 feet, moderate infection up to 10 feet, and occasional infection up to 20 feet. Evidently the practice by some growers of leaving the old stems in place over winter, for the purpose of snow retention, involves a serious risk of communicating stem spot to the succeeding crop.

Varietal Susceptibility

The marked susceptibility of the variety Avalanche has been mentioned. Mon. Bastien Le Page, a discarded commercial variety, was the only peony in a small variety plot maintained at this Station that developed stem spot in 1939. Dr. J. J. Styer of Concordville, Pennsylvania, is the authority for the statement that weak-stemmed varieties, including most reds, and all medium or dwarf growers are susceptible. The varieties Festiva Maxima and Mon. Jules Élie, which are vigorous and thick-stemmed, are but little affected.

SUMMARY

A red-spot disease of the stems, foliage, and flowers of peonies is widely distributed in commercial plantations, but is of infrequent occurrence in a severe form. It sometimes seriously disfigures the plants and may destroy their value for flower cutting.

Its etiological connection with *Cladosporium paeoniae* has heretofore been suspected but not definitely established. It also has been confused at times with the disease first described as anthracnose, but the cause of anthracnose has never been definitely established.

In the search for the cause of stem spot it was found that isolates of *Glocosporium fructigenum* from peony and from *Rubus* may infect peonies as wound parasites. Two other fungi, *Pezizella lythri* and a budding fungus, not further identified as yet, are also pathogenic to peonies, and may cause distinctive stem and leaf diseases. *Cladosporium paeoniae* is considered the principal etiological factor, its restricted development on young stems being due probably to its low virulence on tissues that are in active growth. The profuse character of infection, even though the lesions are small, makes this stem spot a significant disease on peonies grown for flower cutting.

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CROWN GALL OF PEACH IN THE NURSERY

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INTRODUCTION

Crown gall (*Phytopomonas tumefaciens* Smith and Townsend) is one of the most serious diseases on peach in the nursery. In the United States, the disease is particularly prevalent and severe in some sections of the southern and western States. No data are available for estimating the losses occa-

sioned by it, but, where the disease is prevalent, 50 per cent of the trees frequently are discarded, and brush heaps containing thousands of trees having galls like that shown in figure 1, A, are a not uncommon sight. Literature references to this disease in the United States begin about 1890, but undoubtedly the disease occurred many years before that time. Butz¹ in 1902, cites instances where entire blocks of nursery peach trees were destroyed because of crown gall.

Despite the large financial losses caused by this disease, no serious attempts at control have been reported. The standard recommendations have been based on the principle of rotation with crops of nonsusceptible plants, but no reports on the results of this method have been found in the literature.

Limited observations indicate that two important characteristics of this disease appear to be (1) the relatively greater severity in regions known to have alkaline soils, and (2) the localization of the majority of the galls at the region of the root-stem junction, *i.e.*, at the "crown" of the roots. These factors were taken into consideration in seeking means of control by attempting to devise methods whereby (1) noninfested soils could be maintained in that condition; (2) infested soil could be rid of infestation; and (3) the tissue of the seedling could be protected at the region most vulnerable to infection.

PRELIMINARY OBSERVATIONS AND EXPERIMENTS

The effect of the hydrogen-ion concentration of the soil on the amount of crown-gall infection had been investigated, and the results² indicated that liming a relatively acid (pH 5.5) soil greatly increased the amount of infection. This experiment has been repeated and will be discussed under the experiments of 1939.

In connection with the question as to why there is a rather general localization of galls at the region of the root-stem junction, as shown in figure 1, A, peach pits were germinated in the greenhouse and observations made on early stages of growth. It was found that small lesions, probably due to bruising, were present, particularly on the main axis near the cotyledons, in numbers sufficient to account for the presence of numerous galls at this region if these wounded areas became infected. Figure 1, B, illustrates a germinating seed before the cotyledons have emerged from the stony coat (endocarp). Although many pits do not have such a pronounced projection, it is apparent that comparatively slight pressure by the pointed tip of the hard seed coat would result in injury to the very tender tissues of the seedling. Figure 1, C and D, shows wounds that occurred incident to sprouting. That such wounds might serve as infection courts was indicated by the large percentage of seedlings that exhibited galls at the

¹ Butz, G. C. Crown gall. Pennsylvania Agr. Exp. Stat. Ann. Rpt. Part 2 1901/02: 405-414. 1902.

² Siegler, E. A. Relations between crown gall and pH of the soil. Phytopath. 28: 858-859. 1938.

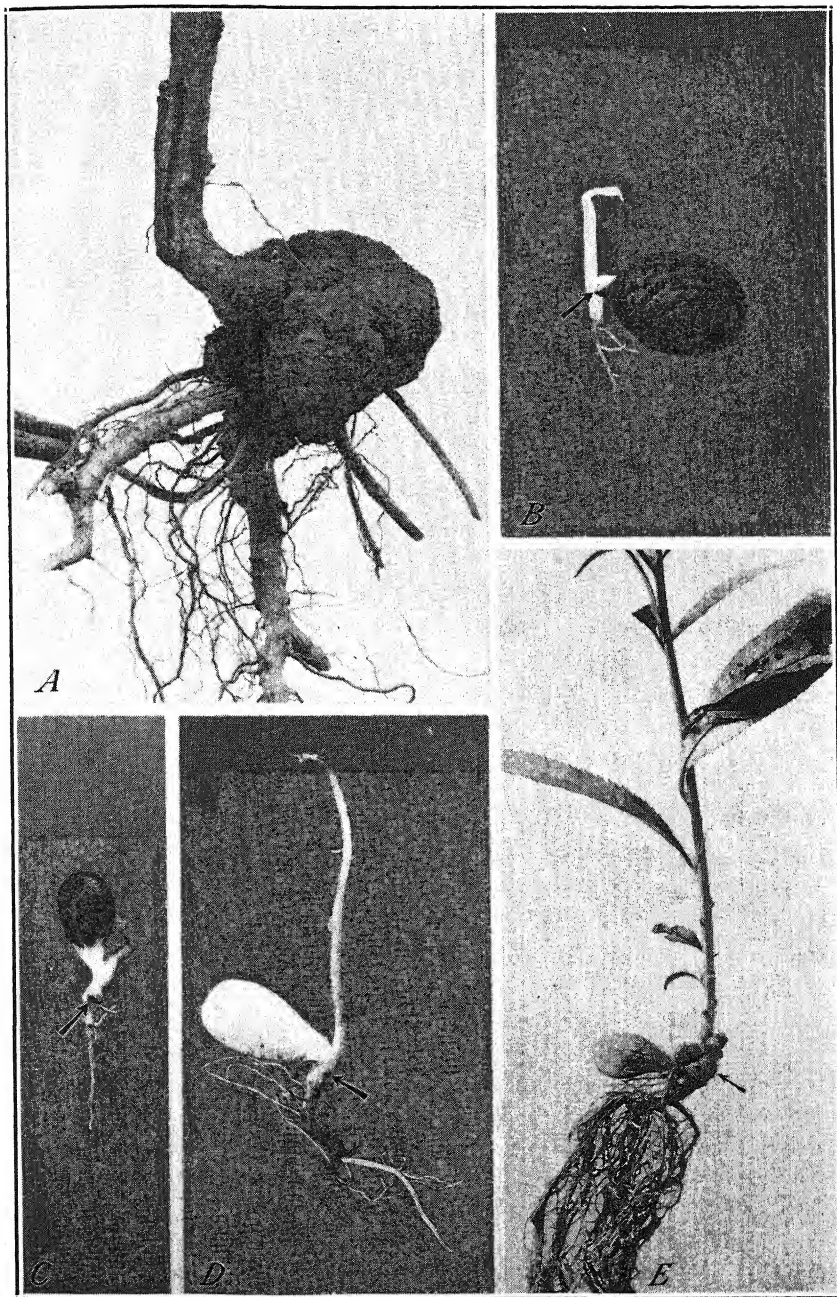


FIG. 1. A. Typical crown gall on 1-year budded peach tree. B-D. Stages in germination of peach. In B, note possibilities for injury to the tender tissues at the region near the cotyledon petioles by the stony endocarp. In C and D, note lesions incident to normal germination. E. Gall on young seedling resulting from natural infection.

cotyledonary region, as shown in figure 1, E, when pits were planted in soil infested with the crown-gall organism.

Preliminary experiments in control, therefore, were made in an attempt to protect the tender tissues of the germinating seed from infection. The seeds in early stages of germination were treated with various antiseptics and disinfectants, including calomel, mercuric chloride, thymol, formaldehyde, "formofume," sulphur, sodium hypochlorate and cuprous oxide. The results of these preliminary experiments were not conclusive because of great variability among the several treated and control plots. However, soil treatment with sulphur and seed treatment with calomel were deemed worthy of further trial.

The more extensive experiments conducted in 1939 were planned mainly to observe the effect of varying the pH of the soil and the effect of applying a disinfectant to the pits, on the amount of infection on 1-year seedlings.

Materials and Methods

Hydrated lime was used when it was desired to make an acid soil alkaline. Sulphur (commercial dusting) and ammonium sulphate were used in an attempt to acidify the soil in certain test plots that had been previously made alkaline by liming.

Calomel (U. S. P.) was used on the pits, which were comparatively free from dirt, at the rate of 4 oz. to 1 gal. of water. The pits were held in an open-mesh cloth bag and dipped in the well-stirred suspension for several minutes to permit a thorough coating. They were then permitted to surface-dry before planting.

The soil in every plot, including the "no treatment" or "check" ones, was artificially inoculated by pouring heavy water suspensions of the crown-gall organism into a 6-inch-wide shallow furrow in which the pits were to be planted. The manipulations were such as to insure a wetting of the soil with the inoculum to a depth of approximately 2 in. below the surface of the soil in the furrow in which the pits were to be planted.

Procedure

In the plots to be tested for the pH effect (Experiment 1), the limed plots received an application of hydrated lime in a shallow 6-inch-wide furrow, and 8 days later all plots were inoculated with the organism.

All the plots to be tested for the effect of calomel, sulphur, and ammonium sulphate and their "check" plots (Experiment 2) were first given an application of lime, and, 8 days later, were inoculated with the organism. In the plots treated with sulphur and ammonium sulphate the chemicals were sprinkled directly onto the pits in the furrow.

The pits were planted in November, 1938.

RESULTS

The details of experiment No. 1, designed to test the effect of the pH of the soil on the amount of crown-gall infection, are shown in table 1.

TABLE 1.—*Comparison of the amount of crown gall on 1-year peach seedlings grown in (1) acid and in (2) alkaline soil*

Row	Treatment	Number of trees	Number of galled trees	Percentage galled trees	pH of soil
1	None	1291	100	8	5.8
2	Limed	1168	713	61	8.5
3	None	835	91	11	5.9
4	Limed	1321	754	57	8.5
5	None	1135	109	10	5.8
Total	None (3 rows)	3261	300	9	5.8
"	Limed (2 rows)	2489	1467	59	8.5

a All pH determinations were made by W. F. Kosar on a quinhydrone electrode.

The results of this experiment confirm those of the preceding year and furnish additional evidence that liming an acid soil apparently makes for conditions favorable for growth of the organism and results in an increased amount of crown gall.

The seedlings in the limed rows made, on an average, slightly better growth (approximately 2 inches) and had slightly darker foliage than those on the non-limed rows. When this condition became apparent, a light side dressing of nitrate of soda was applied to 250 of the seedlings in Row 1. As anticipated, there was a prompt growth response and, by digging time, these trees in this plot were as large as those in any of the limed rows. The total stand was approximately 50 per cent.

In experiment No. 2, an attempt was made to control crown gall by (1) the use of calomel on the pits and by (2) applications of sulphur and ammonium sulphate to the soil. The field plan consisted of two adjacent rows, each row containing 16 plots, 12.5 ft. long. Each plot contained 150 pits and, it will be recalled, the soil in all the plots in these two rows had been limed and then inoculated with the crown-gall organism prior to planting the pits.

The data on the experiment (Table 2) are arranged so that the relative positions of the plots can be readily visualized; the lots that were opposite each other, but in adjacent rows, are on the same parallel lines.

As shown in this table, the stand of trees was very uniform, with the exception of lot 8. In this experiment excellent control was secured by the use of calomel alone (6 per cent galled trees in the 4 plots), as compared with 71 per cent galled trees from the adjacent nontreated check plots 1, 11, 18, 28. Typical trees, classed as clean and galled, are shown in figure 2, A and B, respectively. The fact that the trees in row 2 made on an average approximately 2 to 4 inches more growth and had darker foliage than the trees in row 1 is not shown in the table. The better growth was attributed to leachings from manure that had been spread on higher ground about 8 feet away from row 2. As might be anticipated, the height and caliper of the trees were greatest in all the plots treated with ammonium sulphate. The total stand was 56 per cent.

In contrast to the effect of the calomel treatment alone, this treatment

TABLE 2.—Comparison of the amount of crown gall on 1-year peach seedlings when the pits were treated with calomel and the soil treated with sulphur and ammonium sulphate

Row No.	Lot No.	Treatment (after liming)	Number of trees	Percent-age galled trees	pH of soil	Row No.	Lot No.	Treatment (after liming)	Number of trees	Percent-age galled trees	pH of soil
1	1	None	87	85	8.8	2	17	Calomel	67	18	8.8
"	2	Calomel	95	0	9.0	"	18	None	76	87	9.0
"	3	None	94	70	8.9	"	19	Ammonium sulphate ($\frac{1}{2}$ lb.)	85	87	8.8
"	4	Sulphur ($\frac{1}{2}$ lb.)	76	40	5.0	"	20	None	82	90	8.8
"	5	None	86	71	9.0	"	21	Calomel and sulphur ($\frac{1}{2}$ lb.)	88	48	3.9
"	6	Ammonium sulphate ($\frac{1}{2}$ lb.)	86	69	9.0	"	22	None	82	85	9.0
"	7	None	96	69	9.0	"	23	Sulphur ($\frac{1}{2}$ lb.)	99	73	8.2
"	8	Calomel and ammonium sulphate ($\frac{1}{2}$ lb.)	56	38	8.5	"	24	None	87	75	7.4
"	9	None	92	76	8.8	"	25	Ammonium sulphate ($\frac{1}{2}$ lb.)			
"	10	Sulphur ($\frac{1}{2}$ lb.)	81	49	6.1	"	26	None	94	86	8.3
"	11	None	91	62	8.9	"	27	Calomel	91	1	8.7
"	12	Calomel	80	1	8.8	"	28	None	83	53	8.9
"	13	None	87	71	9.0	"	29	Sulphur ($\frac{1}{2}$ lb.)	85	22	3.8
"	14	Calomel and ammonium sulphate ($\frac{1}{2}$ lb.)	83	0	8.0	"	30	None	83	52	8.7
"	15	None	97	54	8.7	"	31	Calomel and sulphur ($\frac{1}{2}$ lb.)	77	45	7.9
"	16	Ammonium sulphate ($\frac{1}{2}$ lb.)	73	52	8.5	"	32	None	72	53	8.3
Total calomel (4 plots)			333	6							
Checks (4 plots)			337	71							

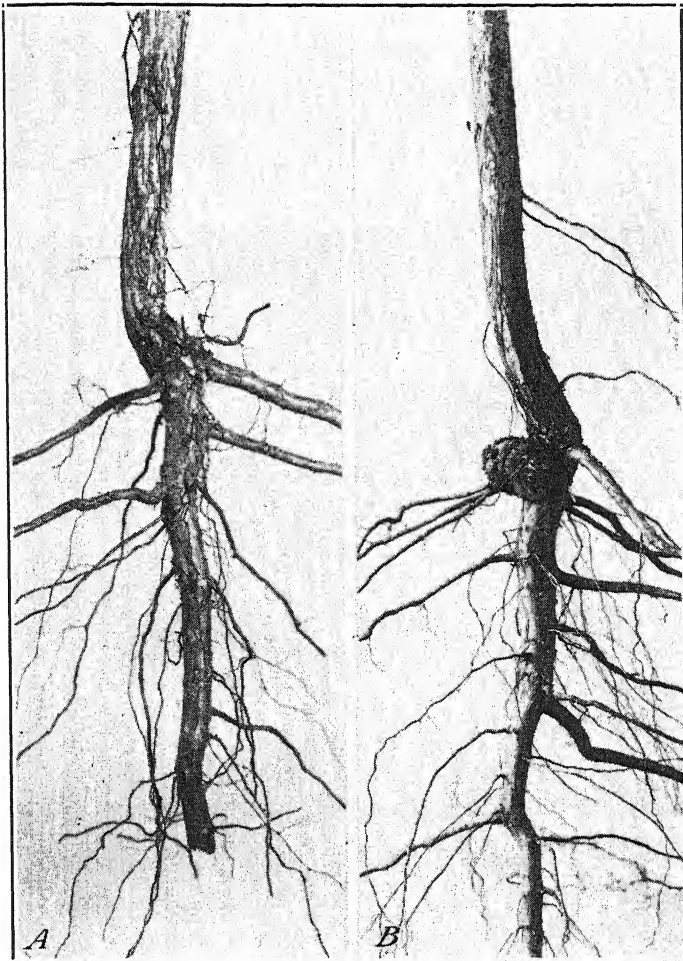


FIG. 2. One-year peach seedlings typical of those in experiments, showing the characteristic "bend" at the root-stem junction. Note tap roots in comparison with branched roots (Fig. 1, A). A. Typical of seedlings classed as "clean." B. Typical of the seedlings classed as "galled." Of the galled trees, 90 per cent had galls located at the "bend" at the root-stem junction.

in combination with either sulphur or ammonium sulphate and the plots receiving the latter materials singly, failed to yield consistent results. So many factors may be involved in these particular treatments that a discussion of them is not warranted at present.

As indicated by the pH readings of the so-called nontreated plots, it would appear that the application of lime resulted in a degree of alkalinity seldom encountered in nurseries. The pH of the land in the immediate vicinity of these two rows was approximately 5.7. Attention is called to the fact that all the pH readings were made from composite samples taken at the time the seedlings were dug, and that the readings above pH 8.0 are not considered reliable. The fact that sulphur particles were abundant

in the sulphur-treated plots at the time of sampling would tend to explain discrepancies in pH readings, as exemplified in plots 23 and 29. Applications of ammonium sulphate had no residual acidifying effect 10 months later. It is possible that applications with this material when the pits are cracking in the spring might result in effective acidification for a short period.

DISCUSSION

The results of these experiments are encouraging for the practical control of peach crown gall in the nursery. Obviously, repeated tests under varying conditions should be made before the results are considered conclusive. The experiments that showed an increase in crown gall as a result of liming an acid soil have confirmed the results obtained in the preceding season and, therefore, are considered more conclusive. In regions where the soil is heavily infested with the crown-gall organism a treatment of the pits with calomel can be made with negligible expense and is at least worthy of a trial.

No data are available on the question as to how closely soil infestation is limited to soils near to or on the alkaline side, or, more especially, as to occurrence of heavy infestation in relatively acid soil. It is hardly necessary to point out that failure to secure satisfactory infestation artificially in an acid soil does not warrant the conclusion that this same soil might not become infested by natural means. However, the known proclivities of other pathogenic soil organisms make it permissible to assume that the presence and the virulence of the crown-gall organism may be influenced by the pH of the soil. Recently, Hornbostel³ has reported that certain organic mercury compounds have greater bactericidal effect on the crown-gall organism when used in media of comparatively low pH values, and Sherbakoff⁴ has reported experiments on the use of sulphur as an acidifying agent to control "true crown gall" on apple grafts.

In these experiments the attempts to change radically the pH of soil which had been made alkaline by liming, yielded inconsistent results. Further experimentation is necessary to learn if the amount of infestation occurring in alkaline soils actually will be appreciably reduced if, by certain treatments, these soils can be made relatively acid. The important question as to the proper alterant for any given soil lies beyond the scope of this report.

In situations where the organism may persist in the soil despite all efforts to eliminate it, the problem of control is mainly concerned with protecting the peach seedling where and when it is most vulnerable to infection. Field observations and preliminary experiments indicate (1) that a large proportion of trees are attacked about 2 inches below the ground

³ Hornbostel, W. Die Beziehungen zwischen Bodenreaktion und Wirkung quecksilberhaltiger Bodenentseuchungsmittel auf den Wurzelkropferreger *Pseudomonas tumefaciens* Smith et Townsend. *Ztschr. f. Pflanzenkrank.* 49: 77-93. 1939.

⁴ Sherbakoff, C. D. Effect of soil treatment with sulphur upon crown gall in nursery apple trees. *Phytopath.* 15: 105-109. 1925.

level, which is at the general region of the root-stem junction, and (2) that severe mechanical injury to the emerging root frequently occurs in early stages of seed germination, causing not only many wounds but frequently the destruction of the growing point, as a result of which many trees exhibit branched roots instead of a normal tap root. The large size and general characteristics of the galls, usually encountered in nursery trees, also indicate that they are 2 years old. Therefore, although other supporting data are not at hand as proof, it can be assumed that wounds at the top or proximal part of the young root system serve as important infection courts for the organism, and that control measures, predicated on this assumption, should be concerned with attempts at (1) elimination of such wounds and (2) protection of the tissue of the young seedling by the antiseptic or disinfectant action of a suitable material. In some sections of the country the practice of planting the sprouted seed in early spring undoubtedly results in a considerable amount of wounding in comparison with the amount that results when the seed is permitted to germinate "in place." In these sections it may be found practicable to hold the seed in a dormant but after-ripened condition in cold storage, so that the pits will not be "cracked" when planted.

The results obtained in these experiments with the use of calomel as a protectant are highly encouraging, but it should be emphasized that these experimental plantings differ in many features from conditions in the commercial peach nursery. These experiments were designed to establish principles for control and, as such, have their value, but such clean-cut results under commercial conditions would not necessarily be obtained. For example, it will be recalled that in these experiments the inoculum was applied only in the immediate vicinity of the pits. There was apparently very slight diffusion of the organism in the soil because all of the galls were confined to the main axis of the root in a region about 2 inches in length, beginning at the root-stem junction; no galls were found on the smaller lateral roots. Moreover, despite the fact that the seeds were planted in the fall and germinated "in place," some wounding undoubtedly occurred; but injury, sufficient to kill the growing point of the young root was evidently infrequent, because practically all of the seedlings had tap roots. By count, however, 90 per cent of the galls on the affected seedlings were located at the "crook," which is formed at the region of the root-stem junction, due to curvatures occurring in early stages of germination. This supports the field observations concerning the location of the majority of the galls and demonstrates the susceptibility of the tissues at this region, presumably while they are still soft and succulent, if not actually wounded.

The fact that the seedlings in these experiments were dug at the end of the first growing season should also be taken into consideration in evaluating these results and in forecasting their applicability to commercial practice where the roots remain in the ground one year longer. Regardless, however, of the number of new infections that may occur during the

second season, it is apparent that protection up to that time is a prerequisite for control.

CONCLUSIONS AND SUMMARY

Crown gall (*Phytomonas tumefaciens* Smith and Towns.) is, in the nursery, one of the most serious of peach tree diseases. The majority of the galls are located at the region of the root-stem junction at the crown of the root system.

The disease is very prevalent in regions where it is the practice to plant the seed after it has sprouted. This practice undoubtedly results in injury more severe than when the pits are planted in the fall and thus are permitted to germinate in place. Even in the latter procedure, however, numerous small lesions occur on the tender tissues during the very early stages of growth. Presumably, these lesions serve as infection courts; but, in any event, the tissues of the roots, particularly in the general region of the root-stem junction, are very susceptible to infection.

Another factor in the etiology of this disease is the general prevalence of the organism in regions in which the soils are relatively alkaline.

The experiments reported here were concerned mainly with securing confirmatory data on the effect of the pH of the soil on the amount of infection and with attempts to lower the pH of soils, made alkaline with lime, by applications of sulphur and ammonium sulphate. In addition, attempts at control were made by dipping peach pits in a heavy suspension of calomel before planting in an endeavor to protect the tissues of the seedling from infection during the early stages of germination.

The results of the experiments furnish additional evidence that a much larger amount of infection occurs when alkaline soils are artificially inoculated than when acid soils are artificially inoculated. The amount of infection was 59 per cent in the limed plots and 9 per cent in the nonlimed plots.

As a result of one season's trial the plots containing the calomel treated pits showed 6 per cent infection in comparison with 71 per cent on 4 control plots.

Regardless of the many factors that should be given consideration in evaluating these results and of the precautionary statements that naturally should qualify the results of preliminary experiments, it is believed that this attempt to establish the important factors incident to infection should eventually lead to adequate control measures.

In view of these results, it would seem advisable to avoid the excessive use of lime on soils in peach nurseries where crown gall is a factor. Obviously, an acid condition as is compatible with satisfactory growth is desired. In addition, treatment of the hard, uncracked pits, with a strong water suspension of calomel (4 oz. to 1 gal.) at planting time is worthy of a trial to test the efficacy of this treatment under various conditions.

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THE INHERITANCE OF IMMUNITY FROM MILDEW (*BREMIA LACTUCAE*) IN LETTUCE

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Milbrath (4) was the first investigator to point out the serious damage to the commercial lettuce crop caused by downy mildew (*Bremia lactucae* Reg.). He stated the "climatic conditions in California are favorable and conducive to the growth of *Bremia lactucae* in the field." This statement is particularly true of the Salinas-Watsonville area, which is the State's major lettuce-producing region. Another troublesome characteristic of the disease is the fact that it continues to develop in shipment. As a result, lettuce attacked by mildew arrives on the markets in poor condition.

Milbrath tested a number of varieties, and found considerable difference in susceptibility to mildew among them. The New York variety proved the most susceptible; Hanson and Iceberg were more or less resistant; none of the varieties tested was immune from the disease.

In 1923, the senior writer (2) reported that a large number of varieties had been tested, and 9 were found to be immune from mildew in both California and Florida. These 9 varieties were of European origin, and, in general, unsuited to cultivation under California conditions. They were crossed with the very susceptible, but commercially popular, variety New York. All first generation hybrids were immune from mildew. Segregation in F_2 closely approximated 3 immune plants to 1 susceptible, suggesting that immunity from the particular physiologic race of *Bremia lactucae* involved was governed by a single dominant gene.

In a series of inoculation experiments on the 9 varieties mentioned above, Jagger and Chandler (3) were able to show very definitely the existence of at least 4 distinct physiologic races of the fungus; race 1, found at Sanford, Florida, and Chula Vista, California; race 2, found in England; race 3, in the Imperial Valley, and race 4, in the Salinas Valley, California. One variety, *Romaine blonde lente a monter*, obtained from France seemed immune from all forms of the fungus encountered. This variety, according to Vilmorin-Andrieux & Cie (9), originated in Southeastern France. It is a typical Cos type lettuce, with pale green, elongated, spatulate leaves. This variety was crossed with several strains of New York or "Iceberg" type lettuce. Through selection and further backcrossing to the New York type, a number of commercially desirable strains resistant to the then known physiologic races of *Bremia lactucae*, and superior to the original New York variety, were released to the industry (Imperial F, Imperial D, Imperial 152, Imperial 615, Imperial 847, etc.).

In 1932, another physiologic race of *Bremia lactucae* appeared in the Salinas Valley, for all of the strains listed above as immune were attacked

¹ Deceased February 16, 1939.

by the parasite. In an extensive series of tests 2 varieties and a selection of *Lactuca scariola* from Russia proved immune from physiologic race 5 of *B. lactucae*. Again, the immune varieties were of European origin; two were primitive, as far as heading qualities were concerned, and commercially useless.

In this paper, data are presented indicating 1, that immunity from physiologic race 5 is dependent upon a single dominant gene; 2, that it is possible to combine genes for immunity with good commercial qualities in lettuce, by crossing, backcrossing, and selection; 3, that genes for immunity are found in the more primitive, non-heading or loose-heading types of lettuce.

MATERIALS AND METHODS

Two types of lettuce immune from mildew were used in these experiments. Their source and description follow.

1. *Grosse blonde d'hiver Bourguignonne*. Obtained from Vilmorin-Andrieux & Cie. A butter-head type. Leaves, smooth with regular margins; plant and head, entirely light green; heart, buttery yellow. Similar to the variety Unrivalled, but probably a little darker green and possibly a little larger. Under our conditions it "tip burns" badly, but is immune from mildew.

2. *Lactuca scariola*.² Obtained through the division of Plant Exploration and Introduction from Russia (P.I. No. 104854). Strong, vigorous plants with pinnately lobed leaves; older leaves, bristly hispid along the midvein; glaucous otherwise; the younger leaves are entirely smooth; no red pigment in either stems or leaves. The young plants form a small rosette of leaves and immediately produce 4 or 5 side shoots, the latter becoming seed stalks of uniform height (24 to 30 in.).

The French variety and *Lactuca scariola* were used directly in the crosses reported in this paper. In the last 3 families listed in table 1, a homozygous resistant segregate derived from an original cross between a plant of *L. scariola* and a susceptible type, with several intervening generations of selection and backcrossing, was developed as a parent for further crosses. It is important to note that resistance in this case originated with the plants of *L. scariola* from Russia.

The susceptible varieties used in these experiments were Imperial F, Imperial D, Imperial 615 and Imperial 847. They are commercial sorts of the "Iceberg" type, and were developed and released by the U. S. Department of Agriculture. At the time of their release, physiologic race 5 had not appeared, and they were immune from the forms of *Bremia lactucae* then present. They are firm, well-folded, and of good quality when grown under conditions to which they are adapted.

Seed for plants to be scored for resistance and susceptibility was planted in the seed bed and the young plants were inoculated with a water suspension of spores of the fungus as soon as the cotyledon leaves became fully expanded. Inoculum was prepared by washing spores from mildew-infected plants. Small cultures of such plants are maintained continuously in order to have a readily available supply of the pathogen for inoculation purposes. After the results of the first inoculation became apparent, the susceptible plants were removed and the remaining plants again inoculated. This second inoculation made it fairly certain that very few potentially mildew-susceptible plants escaped inoculation.

² The evidence is quite conclusive that the cultivated varieties of lettuce have been derived from *Lactuca scariola* (11).

RESULTS

From crosses made between susceptible commercial varieties and homozygous immune ones, immune F_1 plants were obtained. The data obtained for segregation of mildew immunity in the F_2 progenies from these crosses are presented in table 1.

TABLE 1.—Segregation for mildew immunity in F_2 ^a

Cross	Family No.	Diseased	Healthy	Total
F × French variety	3158	147	436	583
D × <i>Lactuca scariola</i>	4502	143	470	613
847 × resistant plant	40018	93	257	350
847 × " "	13279	71	224	295
615 × " "	13247	42	128	170
Totals		496	1515	2011
Calculated (3:1)		502.75	1508.25	

^a Deviation = 6.75; $\chi^2 = 0.12646$; range = 0.00 – 3.841.

The data obtained from the 5 families listed in table 1 have been subjected to the χ^2 test for homogeneity of the individual families. The results indicate that the departures from the theoretical are within the limits of random sampling. The χ^2 test for goodness to fit (3:1) has been applied to each family, independently, and to the totals. Insofar as data on the parents, the F_1 and the F_2 , are concerned it appears that the hypothesis of a single dominant gene accounts satisfactorily for the observations.

The F_3 data are not extensive, but seem sufficient to support the single-gene hypothesis. Five F_2 plants, immune from mildew, from the cross D × *Lactuca scariola*, were tested in F_3 . All proved to be heterozygous (Table 2).

TABLE 2.—Segregation for mildew immunity in F_3

Cross	F_2 Phenotype	Segregation in F_3			χ^2
		Diseased	Healthy	Total	
F × French variety	Diseased	50	50
F × " "	"	8	8
D × <i>Lactuca scariola</i>	"	55	55
D × " "	"	15	15
D × " "	Immune	1	4	5	0.06666
D × " "	"	16	42	58	0.20689
D × " "	"	8	28	36	0.14815
D × " "	"	12	37	49	0.00680
D × " "	"	9	31	40	0.13333

In many cases young seedlings, infected with *Bremia lactucae* are weakened, and die in the seedling stage. With some care to avoid infection by secondary organisms it is possible to raise mildew-infected plants to maturity. Mildew-susceptible plants were selected out from several F_2 families; the progeny from these plants were homozygous for mildew susceptibility (Table 2).

In table 3 are summarized the test-cross data. Test-cross matings were not made to the susceptible parent because it is often difficult or even impossible to determine whether a particular individual is the result of a cross or of self pollination, except by the use of markers. In testing 4 of the F₁ progenies we have made use of a homozygous susceptible plant with a border of red pigment around the leaf edge as a pollen parent, mated to the heterozygous F₁ plants. All cross-pollinated individuals should show red pigment of somewhat the same nature as the pollen parent. In the latter case a typical plant of the variable species *Lactuca scariola* was used. By means of this technique it is a comparatively simple matter to eliminate the self-pollinated plants. The data of table 3 have been subjected to the same tests described for table 1. The results do not deviate significantly from the expected 1:1 ratio.

TABLE 3.—*Segregation for mildew immunity in test cross*^a

Test cross	Family No.	Diseased	Healthy	Total
F ₁ (Imp. D × <i>L. scariola</i>) × red edge suscept.	33570	19	35	54
F ₁ (Imp. 615 × resistant) × "	33578	21	27	48
" " × " "	33580	30	26	56
F ₁ (Imp. 847 × resistant) × " "	33581	28	30	58
" " × speckled red suscept.	33584	3	4	7
F ₁ (Imp. 615 × resistant) × " "	33586	5	5	10
" " × " "	33588	10	13	23
F ₁ (Imp. D × <i>L. scariola</i>) × <i>L. scariola</i> .(suscept.)	33611	3	2	5
Totals		119	142	261
Calculated (1: 1)		130.5	130.5	

^a Deviation = 11.5; $\chi^2 = 2.0268$; range = 0.00 – 3.841.

DISCUSSION

There are two facts established by this investigation that merit further discussion, since they are of general genetic significance. They are: 1. The gene for immunity is dominant over its allele for susceptibility. 2. The plants with the dominant genetic complexes are of European origin.

The 9-chromosome species of *Lactuca* (Babcock, *et al.* (1)) are, with one exception, of European origin. Cultivated lettuce and the closely related species, *L. scariola*, belong to this group. The two facts mentioned above would seem to support Vavilov's contention (8) that the greatest diversity in form of a cultivated species is found in the vicinity of its origin, and that, during the spread of a species toward the boundary of a region, the recessive forms are singled out for survival. Conversely, the proportion of dominant genes is greater in the immediate vicinity of the center of distribution.

Of equal significance may be the fact that the immune types are more or less primitive, or nonspecialized. The highly developed, specialized heading types, *i.e.*, New York, etc., are, without exception, carriers of the recessive genes for susceptibility.

The dominant gene for immunity from mildew in lettuce parallels very closely in origin and behavior the *Fu* gene for resistance to *Fusarium* wilt described by Wade, *et al.* (10) in peas. The similarities are obvious; the genes for resistance or immunity are dominant, and are found in relatively unspecialized types; forms with dominant genetic complexes are found near the center of distribution of the species in question. It, however, is not true that all of the primitive types of lettuce either cultivated or wild (*L. scariola*), carry the dominant genes for immunity.

The recent work of Schultz and Röder (5) supports our observations that the more primitive types of lettuce carry the dominant genes for immunity. In their very extensive trials at the Experimental and Research Institute for Horticulture in Germany, they found 3 varieties of the general type of May King (May King, May King Forcing, Bottner's Forcing), which appeared to be very resistant to if not immune from the disease. These varieties are of the early forcing type, making loose, spongy heads and having buttery-texture leaves. The senior writer has found May King immune from most of the physiologic races encountered in this study, but it was susceptible to certain races in England, and in Imperial Valley, California.

Schweizer (6) has shown that there is considerable physiological specialization within *Bremia lactucae*; that is, spores from one host infect only the same host or other species of the same genus as the host. In no case was he able to cross-infect to species of another genus. By means of statistical methods, Schweizer was able to distinguish what he termed "small morphological species" within *B. lactucae*. Schultz and Röder (5) have isolated 2 physiological races of *B. lactucae* in Germany. These races were separated on the basis of differential pathogenicity to certain varieties of lettuce.

Stakman (7) lists 4 principal methods that have been suggested to explain the origin of races of phytopathogenic fungi. They are as follows: adaptation, hybridization, heterocaryosis, and mutation.

We have no critical test that would positively discredit the theory of the origin of physiological races in *Bremia lactucae* through adaptation. Indirect evidence, however, seems to indicate that it is highly unlikely. If the parasite had adapted itself to previously immune varieties, these presumably would have "lost their resistance" rather gradually over a series of growing seasons. Actually, the new physiologic races appeared suddenly, and varieties that had been immune were completely susceptible to the new forms.

It is doubtful whether either heterocaryosis or hybridization should be considered as probable methods of origin of physiological races in *Bremia lactucae*, for the reason that the sexual stages of the fungus have never been observed in this locality. Our knowledge of this subject is far from complete; it is entirely possible that a careful examination might disclose sexual reproduction in the life history of the parasite.

That physiological races of *Bremia lactucae* have originated through mutation seems reasonable from circumstantial evidence. The manner in which they first appeared, and the fact that resistance to at least two of them is controlled by single gene differences would indicate that they may have originated through mutation.

These observations make the assignment of producing disease-resistant plants a difficult one. The best opportunity for success seems to be in maintaining a large collection of types of the host species, including primitive types from near the center of origin of the species on the chance that some of them will carry genes for resistance to new physiologic races, as these races are discovered, and become economically important.

From general observations there is no evidence of linkage between genes for immunity, and those for the various pigments, morphological structures, or physiological characteristics of the strains or varieties of lettuce used in this study. For this reason it is a comparatively simple matter to combine the genes for immunity with those for desirable commercial qualities.

SUMMARY

The occurrence of 5 physiologic races of *Bremia lactucae* is recorded. There is evidence of the existence of as many as 6 or 7 races of this fungus that attack cultivated lettuce.

The inheritance of immunity from physiologic race 5 is described in detail. Immunity is dependent upon a single dominant gene.

Dominant genes for immunity have been found only in the more primitive types of lettuce. These types occur in Europe, and presumably come from near the point of origin of cultivated lettuce.

There is no evidence of linkage between genes for immunity and any of those for the various morphological characters found in cultivated lettuce.

It is suggested from indirect evidence that physiologic races in *Bremia lactucae* originate through mutation.

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HYDROXYL-ION CONCENTRATION OF THE SALIVA OF PARTLY DESICCATED BEET LEAF HOPPERS

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The beet leaf hopper, *Eutettix tenellus* (Baker), is the only known vector of the curly-top virus. The virus exists in the body of the insect for long periods (3, 6). Knowledge of the chemical environment of the virus within the leaf hopper is, therefore, of interest. Earlier investigations have shown that the salivary secretions of normal beet leaf hoppers are distinctly alkaline, and that the blood or body fluid is slightly alkaline (4, 1). Bennett and Wallace (3) came to the conclusion that the blood of the insect is the main reservoir of the virus. The phloem of sugar beets, which is a favorable environment for the virus, is slightly alkaline (5).

The object of this paper is to present data on the hydroxyl-ion concentration of the salivary secretions of starved and, consequently somewhat desiccated leaf hoppers, and to correlate the findings with some facts previously observed.

METHODS

In order to determine the equivalents of hydroxyl ions, secreted during feeding by the salivary glands of partly dessiccated leaf hoppers, it was necessary to allow the insects to feed on small drops of a slightly buffered liquid for a definite period.

A buffer solution, consisting of 0.01 normal HCl and 0.09 normal KCl, was diluted 10 times with distilled water to make a feeding solution. The object of diluting the buffer solution was to reduce its buffer capacity and at the same time to increase its pH to a point beyond its most effective buffer range. Sufficient sucrose was then added to bring the concentration to 2 per cent. This feeding solution, freshly prepared, had a pH of 3.07. Calculations from the buffer curve show a maximum buffer value of 1×10^{-5} between pH 3.31 and 3.11 and a minimum buffer value of 1.28×10^{-4} between 4.48 and 5.12. The feeding solution had a buffer value of 3.7×10^{-4} over the entire pH range through which it was used. The feeding solution had sufficient buffer capacity to maintain a constant hydrogen-ion activity, yet the buffer capacity was so low that the addition of an extremely small

quantity of hydroxyl ions would shift the pH considerably. A fresh feeding solution was prepared when the pH was found greater than 3.65 or when evidence of bacterial growth appeared.

Female leaf hoppers, which had been kept without food or water for 18 to 24 hours at room temperature, were placed singly in individual feeding chambers previously described (4). When a leaf hopper in search of food punctured the paraffine membrane, a drop (0.01 cc.) of the feeding solution was placed on the membrane directly over the insect. In this way it was possible almost at will to induce desiccated leaf hoppers to feed.

A leaf hopper was allowed to feed for a definite period of time, then the drop was transferred to another paraffine membrane for the pH determination. The transfer was accomplished by inverting the feeding chamber. In this position the drop would still adhere to the underside of the membrane. Another paraffine membrane, stretched across the mouth of a small vial, was raised until it came in contact with the drop and then lowered. The drop would then adhere to the lower membrane. The feeding chamber was next righted and another drop placed over the leaf hopper whose mouth parts were still protruding through the membrane. In this way a leaf hopper could feed continuously, being interrupted only for about 10 seconds, while the drop of feeding solution was being replaced by a fresh one.

The drops were changed at regular intervals until the leaf hopper refused to feed. By changing the drop at regular intervals it was possible to follow the rate at which the insect injected hydroxyl ions into the feeding solution.

The pH determinations were made alternately (with the quinhydrone electrode) on the drops on which the leaf hoppers had fed and on control drops of the same volume that had stood in the open and under the same conditions for the same length of time. The electrode consisted of a platinum wire ground down until the tip was approximately 60 μ in diameter. A fine capillary tube filled with agar saturated with salt served as the salt bridge.

The pH of the control drops was constant. As many as 20 tests on the control drops would be made during one day. The probable error of the mean for any series of tests made on the same day was never greater than ± 0.04 pH unit.

RESULTS

The pH of the saliva of 75 leaf hoppers was tested in the manner described above. The saliva of most of the insects was tested more than once. Nearly all of the leaf hoppers were tested as long as they would feed, the feeding drops being changed at regular intervals.

As many as 20 tests were made during the feeding period of a single leaf hopper. In these tests the feeding drops were changed every 2 minutes; in other tests the feeding drops were changed every 5 minutes. The points in figure 1 show the pH of the feeding drops after the injection

of hydroxyl ions by 4 leaf hoppers, A, B, C and D, and are typical of the results obtained. Each point represents a drop on which leaf hopper A or B, respectively, fed for a period of 2 minutes, while each point, with one exception (leaf hopper C, drop 6), represents a drop on which leaf hoppers C or D, respectively, fed for 5 minutes.

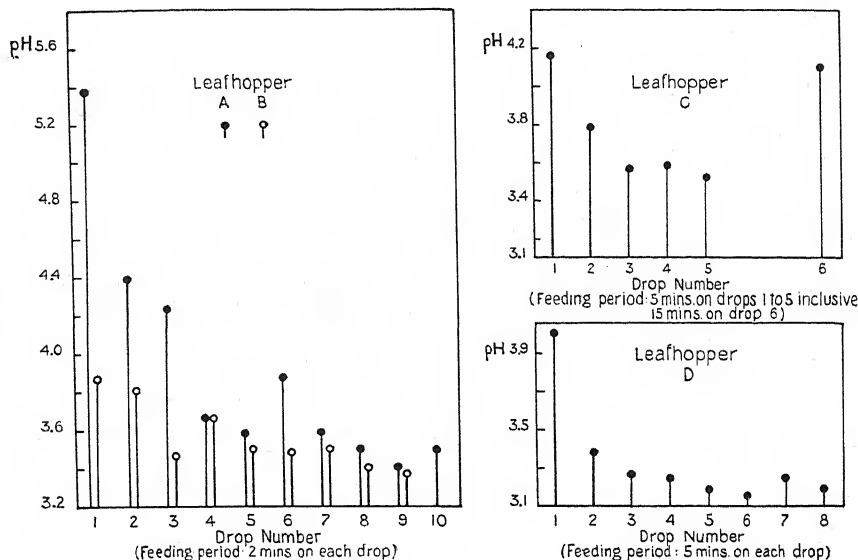


FIG. 1. The change in pH of 4 series of drops upon which 4 leaf hoppers were separately fed. The ordinates indicate the pH of the individual drops at the conclusion of the feeding period. The pH of the drops before feeding started was 3.2 for leaf hoppers A and B and 3.1 for leaf hoppers C and D.

The leaf hoppers were able to increase the pH of the feeding solution in all cases where they were known to have fed, regardless of whether or not they had been previously desiccated. It is evident from the figure, however, that the equivalents of hydroxyl ions injected was generally less in each successive drop. During the latter part of the feeding period when the leaf hoppers were no longer in a desiccated condition hydroxyl ions were still being excreted.

The equivalents of hydroxyl ions injected into each drop of feeding solution was determined from the buffer curve.¹ The pH that would be obtained, if the same number of equivalents of sodium hydroxide were added until, at the point where the buffer value was least, only 0.01 cc. of sodium hydroxide was added between pH determinations. The pH values were plotted against the total volume of sodium hydroxide added. The volume of 0.001 normal sodium hydroxide required to bring 5 cc. of the feeding solution to the same pH value as that of the drop on which the leaf

¹ The pH was determined (with the quinhydrone electrode) on 5 ml. of the feeding solution after each addition of small increments of 0.001 normal sodium hydroxide. The maximum volume of sodium hydroxide added between pH determinations was 0.50 cc. As the buffer capacity decreased, smaller increments of sodium hydroxide were added until, at the point where the buffer value was least, only 0.01 cc. of sodium hydroxide was added between pH determinations. The pH values were plotted against the total volume of sodium hydroxide added. The volume of 0.001 normal sodium hydroxide required to bring 5 cc. of the feeding solution to the same pH value as that of the drop on which the leaf

It is evident from the figure and the data presented in table 1 that the equivalents of hydroxyl ions injected into the first, second, and third drops are large compared to that injected into the remaining drops.

TABLE 1.—*The secretion of hydroxyl ions by partly desiccated beet leaf hoppers while feeding on a slightly buffered sugar solution*

Drop (0.01 cc.)	OH ions secreted into each drop				Calculated pH of drop of water containing same equivalents of OH ions			
	A ^a	Leaf hopper		D ^b	A	Leaf hopper		D
		B ^a	C ^b			B	C	
No.	<i>10⁻¹⁰ Equivalents</i>				<i>p^H</i>			
1	70	44	58	54	10.85	10.64	10.76	10.47
2	58	42	47	23	10.76	10.62	10.66	10.36
3	54	22	36	14	10.73	10.39	10.56	10.14
4	34	33	38	12	10.53	10.51	10.58	10.01
5	30	24	33	6	10.48	10.38	10.52	9.78
6	44	24		3	10.65	10.38	10.77	9.50
7	30	24		12	10.48	10.38		10.08
8	24	18	57	6	10.38	10.26		9.78
9	18	15			10.26	10.18		
10	24				10.38			
Total	368	246	269	130	11.59 ^c	11.39 ^c	11.43 ^c	11.11 ^c

^a Leaf hoppers fed 2 minutes on each drop.

^b Leaf hoppers fed 5 minutes on each drop, with one exception. Leaf hopper C fed 15 minutes on drop number 6.

^c The (calculated) pH that would result if all the hydroxyl ions, secreted by the leaf hopper, were concentrated in one drop (0.01 cc.) of water.

If the leaf hoppers are allowed to feed for longer periods on the same drop of feeding solution, the equivalents of hydroxyl ions injected are generally greater. A typical example of this is shown by leaf hopper C. This insect, after feeding 5 minutes on each of 5 drops, was allowed to feed 15 minutes on the 6th drop.

DISCUSSION

If it is assumed that the saliva of the leaf hopper is diluted 1000 times when injected into a drop (0.01 cc.), then 1×10^{-5} cc. of leaf-hopper saliva² containing the 70×10^{-10} equivalents of base was injected by leaf hopper A into the first drop of feeding solution. If these assumptions are used, calculations show that the total concentration of bases in the leaf hopper's saliva would be about 0.7 normal.

The actual volume of saliva injected into the drop of feeding solution is not known. Nevertheless, the volume of saliva that was injected into each drop contained the number of equivalents of hydroxyl ions shown in table 1.

hopper had fed, was read from the buffer curve. The equivalents of hydroxyl ions required to change 0.01 cc. (one drop) of the feeding solution over the same pH range was then calculated. In this way the equivalents of hydroxyl ions injected into each drop of feeding solution by the leaf hoppers were determined.

² The average weight of a female leaf hopper is approximately 0.0015 gram. If the leaf hopper ejects 1×10^{-5} cc. of saliva (density assumed to be 1.0) into each drop of feeding solution this volume would amount to approximately 0.66 per cent of its weight.

As proteins in general are quite inefficient buffers and their presence in the saliva probably would have little effect on the buffer capacity, it appears that the leaf hopper's saliva is highly buffered with inorganic salts.

It is evident that the pH of the nondiluted saliva would be greater than when highly diluted. From this it appears that the pH of the nondiluted saliva of desiccated leaf hoppers would equal at least the values shown in table 1, or higher. The dissociation of sodium and potassium carbonates and the tertiary phosphates of sodium and potassium are sufficient to account for these pH values.

It seems logical that the total salinity and the pH of the blood of the leaf hopper must be maintained within narrow limits. If this be true, then, as desiccation proceeds, it seems reasonable that water would not be spared to eliminate the salts through the alimentary tract. If the salivary glands are the reservoir for the excess salts of the body fluid, then it would seem logical that the salts would be eliminated most rapidly by ejecting saliva containing the salts during feeding.

Bennett and Wallace (3) found that, when leaf hoppers were kept without food and water 18 hours or longer and then allowed to feed 6 minutes on each of 20 seedling beets consecutively, the percentage of infection was low for the first period, increased somewhat in the second, and rose in the third to a level that was then maintained. They state: "Starvation combined with a certain amount of desiccation may have brought about certain changes that tended to inactivate any virus that might have been held in the salivary glands; hence the usual amount of infection could have been produced only after these conditions were corrected." It may be that the high concentration of hydroxyl ions in the saliva of partly desiccated leaf hoppers results in inactivation of much of the virus then in the salivary glands.

SUMMARY

The saliva of beet leaf hoppers that had been kept without food or water 18 to 24 hours contained a high concentration of hydroxyl ions. Measurements and calculations show that the pH of normal leaf hoppers' saliva is greater than 10 and may reach approximately 11 in the saliva of desiccated leaf hoppers.

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SWEETCLOVER, A PROBABLE HOST OF TOBACCO STREAK VIRUS¹

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(Accepted for publication January 6, 1940)

Streak, a virus disease of tobacco, has been observed in Kentucky since 1923 but until the past three years outbreaks were so rare that it was given but little attention. In 1932 the disease was named and photographs from an outbreak that occurred in 1923 in Marion County, Kentucky, in topped tobacco were published.² In 1933 E. M. Johnson transmitted the virus from an affected plant in the field to a plant growing in the greenhouse by grafting.³ The graft was made July 16 and symptoms developed in a sucker August 15, 1933, thus proving the virus nature of the disease. In 1935 J. Johnson⁴ published a comprehensive study of the disease and also named it streak. During the past 3 years reports of serious outbreaks of the disease have been received from county agents and farmers. Three fields have been seen in which streak was scattered throughout extensive plantings with percentages of 10 to more than 50 in the middle of the fields. Usually, streak is limited to a few plants at the edge of the field or to scattered plants in the field. The worst outbreaks of the disease have been reported from Boone, Pendleton, Grant, and neighboring counties in the extreme northern part of the State, but it is generally state-wide.

The virus of streak is transmitted with difficulty mechanically, if at all, unless very recently invaded necrotic tissue is used as inoculum⁵; and even then the percentage of positive transfers may be low.

Tobacco usually does not live through the winter in Kentucky, and the virus does not appear to be seed transmitted; therefore, it seems obvious that it has some other host, which is at least biennial, and that an insect vector must be concerned.

SWEETCLOVER A PROBABLE SOURCE OF STREAK VIRUS

Sweetclover (*Melilotus alba*) is affected by a virus that we have been unable to transmit mechanically to tobacco. It is characterized by chlorosis and sometimes chlorotic or necrotic ring and line patterns. The virus is commonly observed in small patches of sweetclover and in sweetclover that is pastured or mowed, but in extensive undisturbed plantings the disease usually is not evident. Observations of outbreaks of streak in

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

² Valleau, W. D., and E. M. Johnson, Tobacco Diseases in Kentucky. Kentucky Agr. Exp. Stat. Bull. 328: 111-154. 1932.

³ Unpublished.

⁴ Johnson, J., Tobacco streak, a virus disease. Phytopath. 26: 285-292. 1936.

⁵ This suggestion was obtained from J. Johnson, who used the method in his studies of the streak virus.

Pendleton County, where sweetclover grows along roadsides and in waste areas and is used as a soil-building crop, suggested to the writer in 1937 that there was a relation between outbreaks of streak in tobacco and sweetclover, as, near each field where streak was severe, sweetclover was found. Many observations since that time in northern, central, and western Kentucky have indicated that where streak occurs sweetclover may be expected to be found in the immediate vicinity. Sweetclover is not a common weed in Kentucky, except in the northern part of the State where limestone outcrops. In other parts of the State it is sometimes a roadside weed. Several specimens of streak in Burley tobacco have been received from Burley-tobacco growers in Missouri, and, in each case, inquiry revealed that sweetclover was growing adjacent to the affected plants.

Following the writer's visit to Pendleton County on July 20, 1937, C. E. Bortner called his attention to a severe outbreak of streak in Burley tobacco in a recently established tobacco rotation on the Experiment Station farm at Lexington, where tobacco was growing for the first time in a plot adjacent to 2nd-year sweetclover. Whereas in previous years not over 3 or 4 streak plants had been found any year on the farm in about 50,000 plants, this small plot (150 plants) had 13 streak plants on that date and, in the following 2 years, streak was abundant in comparable plots (Table 1). In 1939, .12 per cent streak developed in the remainder of the rotation series, but 10 of the 15 streaked plants were within a short distance of sweetclover plots.

TABLE 1.—*Streak in Burley tobacco growing adjacent to second-year sweetclover*

Year	Plot no.	No. of streak plants	Percentage streak in plots	Percentage streak in other plots
1936 ^a	0	0.0 ^b
1937	529	25	16.6	.035
1938	629	32	21.3
1939	729	33	22.0	.12 in 13,650 plants

^a No second-year sweetclover was present in the entire series.

^b Counts were made by C. E. Bortner.

The relatively high percentages of streak for each of 3 years, in tobacco growing within 20 feet of sweetclover plants, and the extremely low percentage in the remainder of the series and on other parts of the farm year after year give nearly conclusive proof that the virus was transmitted from the sweetclover. The weed population in the sweetclover would certainly be duplicated on other parts of the farm. It also suggests that the insect vector travels relatively short distances, or, if it travels longer distances, seeks other host plants than tobacco. Infection in tobacco occurs at about the time sweetclover seeds are forming, suggesting that, because of the hardening of sweetclover, the vector is forced to seek other food plants. It is

unlikely that tobacco is one of the preferred hosts of the vector, so that infection on tobacco is probably purely accidental.

That the growing of sweetclover in extensive plantings is not necessarily a menace to tobacco growing in the vicinity is indicated by the following observations: A tobacco grower in Woodford County has, for 18 years, grown tobacco following a rotation of winter wheat, sweetclover, and weeds for about 3 to 5 years. He does not disturb the sweetclover in any way, such as by cutting or pasturing, until it is plowed for tobacco. There are both one- and two-year sweetclover in these fields, as the ground contains quantities of seed. The rotation is excellent because the value of the Burley crop has increased each rotation during this period. Although tobacco is frequently grown near sweetclover, streak plants are extremely rare on two farms handled in this manner, but an occasional affected plant has been found. The sweetclover appears to be relatively free from virus disease. The supposition is that the insect vector, not being disturbed by stock pasturing in the sweetclover, or by mowing, does not move great distances. Consequently, the virus is not spread rapidly and the majority of vectors remain nonviruliferous. When the old sweetclover dies there is an abundance of young plants on which to feed.

In contrast a field of tobacco in the same county was observed a part of which was growing next to a railroad where scattered plants of sweetclover were growing. The grower insisted that the sweetclover be mowed. About 2 weeks later, 29 per cent of the tobacco plants in several rows parallel to the railroad were affected with streak, while the tobacco in the same planting about 50 yards away had only scattering streak plants. Evidently either the mowing or maturity of the sweetclover made it necessary that the vector move and it had migrated into the nearby tobacco, fed, and transmitted the virus.

CONCLUSIONS

These observations, while not conclusive, indicate that sweetclover is probably the host from which an insect vector carries the streak virus to tobacco growing in the vicinity. Roadside plants or scattered sweetclover plants in waste places seem more likely to become infected, and to act as sources from which tobacco may become infected, than extensive sweetclover plantings, if these are not disturbed by grazing or mowing. Sweetclover sowed in fields not well stocked with sweetclover seed might be a source of infection of nearby tobacco because of migration when the sweetclover became tough during ripening. The presence of young clover might prevent migration. It is probable that the destruction of second-year sweetclover in the immediate vicinity of fields to be planted to tobacco, before the tobacco is planted, will result in control in areas where the disease has proved injurious.

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DIAPORTHE VACCINII, THE ASCIGEROUS STAGE OF PHOMOPSIS, CAUSING A TWIG BLIGHT OF BLUEBERRY

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(Accepted for publication January 8, 1940)

INTRODUCTION

In 1936 the writer reported the occurrence of a *Phomopsis* twig blight on the cultivated blueberry, *Vaccinium corymbosum* L., in both Massachusetts and New Jersey.¹ In a later paper² this blueberry twig-blight fungus was shown to be identical with *Phomopsis vaccinii*, the imperfect stage of *Diaporthe vaccinii* Shear, which causes a serious decay in the fruits of the cranberry, *Vaccinium macrocarpon* Ait. The present paper reports the development of the perithecial stage in cultures of the blueberry twig-blight *Phomopsis* and its identity with *Diaporthe vaccinii*.³

SOURCE OF CULTURES

Cultures in which perithecia developed were obtained from the following 3 sources: Subcultures from original isolations made in 1936 from small sterilized pieces of naturally blighted blueberry shoots; the pulp of decayed cranberry fruit; and reisolations of the *Phomopsis* from diseased areas of artificially infected blueberry plants. Cultures were made on cornmeal agar in July, 1938; and, in October, while producing only pycnidia, 17 were placed out of doors at the United State Horticultural Station, Beltsville, Maryland, and not again examined until February, 1939.

All the cultures were then found to have occasional small, thick, stroma-like bodies, black on the exterior, partly or wholly embedded in the substratum (Fig. 1, C, a and b), in which were *Phomopsis* pycnidia and perithecia of the *Diaporthe* type, either together in the same stroma (Fig. 1, B, a and b) or in separate stromata (Fig. 1, A). The occasional formation of pycnidia and perithecia in the same stroma is a cultural characteristic of cranberry isolates of *Diaporthe vaccinii*. Perithecia-bearing stromata continued to form for several months in these cultures, kept in a refrigerator at a temperature of 8° C. Single-ascus transfers from cultures made originally from naturally blighted blueberry shoots later produced the ascigerous stage at room temperature.

In the original description⁴ of *Diaporthe vaccinii*, Shear³ states that

¹ Wilcox, Marguerite S. Notes on blueberry fungi. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 20: 106-107. 1936.

² Wilcox, Marguerite S. *Phomopsis* twig blight of blueberry. Phytopath. 29: 136-142. 1939.

³ Shear, C. L., N. E. Stevens, and H. F. Bain. Fungous diseases of the cultivated cranberry. U. S. Dept. Agr. Tech. Bull. 258. 1931.

⁴ "In stromata on cranberry fruit and in culture, but separate and without any trace of stroma on dead cranberry vines; perithecia on stems grow between bark and wood, with eccentric neck protruding through the bark, nearly hemispherical, 0.3-0.5 by 0.2-0.4 mm.; wall two to several cell layers thick, black, carbonous; on decayed berries perithecia in stromata, with long perithecial necks protruding in all directions from folds of the shriveled berry; in cornmeal agar cultures perithecial stromata are partly embedded, about 1.5-2 mm.

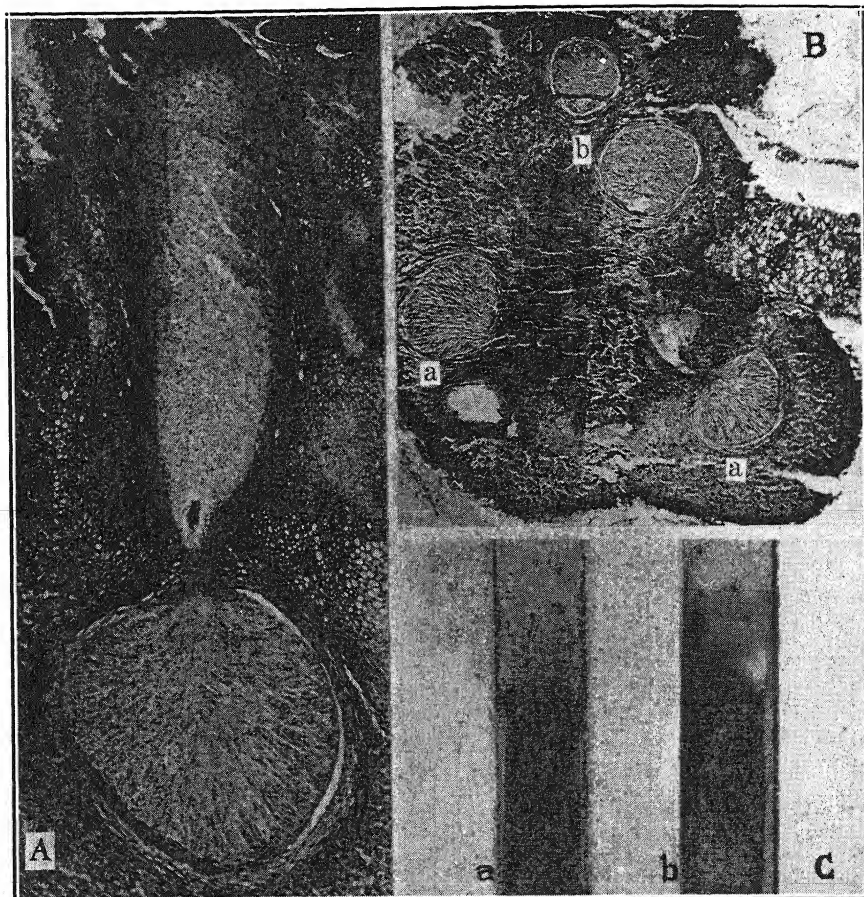


FIG. 1. *Diaporthe vaccinii* in cornmeal-agar cultures. A. Perithecium in stroma embedded in agar. Isolate from naturally blighted blueberry shoot. $\times 135$. B. Cross section of stroma embedded in agar. Reisolated from artificially infected blueberry plant. a. Perithecia. b. Pycnidia. $\times 56$. C. Cultures of *Diaporthe vaccinii*. a. From naturally blighted blueberry shoots. b. From pulp of decayed cranberry fruits. $\times 3$.

the perithecia are formed in stromata on cranberry fruits and in culture, but separate and without any trace of stromata on dead cranberry vines. The ascigerous stage of *Phomopsis vaccinii* has not been found on the blueberry in nature; consequently, it is not known whether the perithecia should be expected to occur separately (not in stromata), as is the case on cranberry.

In cornmeal-agar cultures of the blueberry isolates, the perithecia are definitely stromatic, and both stromata and perithecia appear to be iden-

in diameter, with numerous beaks growing to a length of 0.5 mm.; perithecial necks several cells thick, heavy-walled, black, carbonous, copiously supplied with upward-directed hairs; asci oblong fusoid, sessile, 37–51 by 6.8–11.7 μ , apex thickened and pierced by a narrow pore; spores irregularly biseriolate, ellipsoid, obtuse; 2-celled, slightly constricted at the septum, each cell typically biguttulate, 8.8–11.8 by 2.4–3.4 μ ." (p. 7)

tical with the ascigerous stage of the cranberry *Diaporthe*, as described by Shear, from cultures.⁵ The production of stromata containing both pycnidia and perithecia (Fig. 1, B, a and b) and the elongated thick-walled ostioles copiously supplied with upward-directed hairs (Fig. 1, A) are additional evidence of the identity of the isolates from the two hosts. The asci are oblong, fusoid, sessile with narrow pores, 32–48 by 5.8–9.6 μ ; spores 2-celled, slightly constricted, and biguttulate, 6.4–12.8 by 2.5–4.2 μ (Fig. 2).

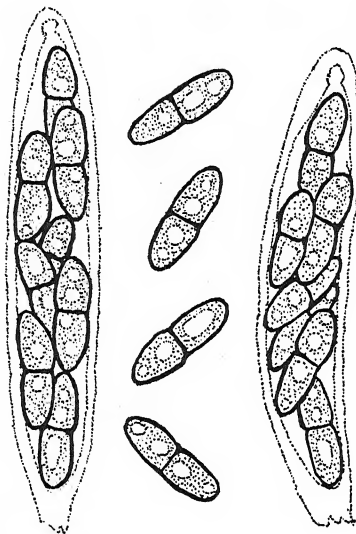


FIG. 2. *Diaporthe vaccinii*. Asci and ascospores from blueberry isolate grown on cornmeal agar. $\times 1360$.

At the same time comparative measurements were made of *Diaporthe vaccinii* from cultures made from the pulp of decayed cranberry fruits. The asci were 35–45 by 6.4–9 μ , and the spores 6.4–11.6 by 3.2–3.8 μ . The asci and spores, the formation of perithecia in stromata, and all cultural characteristics of *Diaporthe* from the blueberry isolates appear identical in every respect with *Diaporthe vaccinii* Shear,⁵ the cause of a decay in cranberry fruits.

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⁵ See footnote 3.

THE RELATIONSHIP BETWEEN VIRUSES OF POTATO CALICO AND ALFALFA MOSAIC

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(Accepted for publication Dec. 5, 1939)

It was observed that potato-calico virus produces symptoms in *Nicotiana glutinosa* L. very similar to those caused by alfalfa-mosaic virus (*Marmor medicaginis* H.)¹ in the same host plant. This observation suggested that the two viruses might be closely related. It is the purpose of this paper to point out the similarities and differences in the symptoms of the two diseases and to report the results of cross protection tests. The investigation showed that the two viruses, although distinct, are indeed closely related.

VIRUS STOCKS

Potato-calico virus was obtained from E. S. Schultz. It is apparently identical with that studied by Porter (5, 6, 7) and Dykstra (2). On the basis of symptomatology, Porter (6) distinguished between the viruses of potato-calico and potato-auricula mosaic (*Marmor auricula* H.). Dykstra (2) likewise distinguished between these viruses and reported further that potato-calico virus is apparently unrelated to the viruses of potato-auricula mosaic and Canada-streak (*M. auricula* H. var. *canadense*, n. var.),² a conclusion which is confirmed in the present paper.

Alfalfa-mosaic virus was secured from H. T. Osborn. It is believed to be the same as or a closely related strain of that studied by Zaumeyer and Wade (10) and Zaumeyer (11) and designated by the latter as alfalfa-mosaic virus 1. The literature is somewhat confused as to whether there are one or two alfalfa-mosaic viruses. Pierce (4) distinguished between the alfalfa-mosaic virus studied by Weimer (8, 9) and that studied by himself and accordingly referred to the viruses as alfalfa viruses 1 and 2, respectively. Zaumeyer (11), on the other hand, considered Weimer's virus and Pierce's virus to be the same and referred both to alfalfa-mosaic virus 1. The writers agree that there is insufficient evidence for distinguishing between alfalfa viruses 1 and 2.

COMPARATIVE SYMPTOMATOLOGY

Both potato-calico virus and alfalfa-mosaic virus were transmitted to several species of plants by the rubbing method of inoculation. In most cases carborundum was employed. Both viruses were readily transferred from young *Nicotiana glutinosa* plants to other plants of the same species. They produced almost identical symptoms after an incubation period of 3 to 4 days.

¹ Latin binomials used in this paper are based on the system of nomenclature in the Handbook of Phytopathogenic Viruses (3).

² It has been shown (1, 2) that the viruses of potato-auricula mosaic and Canada-streak are related. The comparative symptomatology of the induced diseases clearly indicates that the viruses are not identical. For this reason, it is felt that the Canada-streak strain of virus should have varietal rank. The name *canadense*, suggested by the common name, seems appropriate.

N. glutinosa proved to be a useful test plant and a good source plant for both viruses. On beans (*Phaseolus vulgaris* L. var. Early Golden Cluster and Corbett Refugee) and on Black Eye cowpea (*Vigna sinensis* Endl.) they produced the same type of necrotic primary lesion. On beans, lesions sometimes appeared within 24 hours after inoculation. Both viruses produced necrotic primary lesions followed by a systemic streak disease in broad bean (*Vicia faba* L.). On seedlings of Green Mountain potatoes (*Solanum tuberosum* L.), they produced similar symptoms but the symptoms of potato-calico virus were more severe than those caused by alfalfa-mosaic virus. Both viruses caused mottling and necrotic vein-banding in crimson clover (*Trifolium incarnatum* L.) and in red clover (*T. pratense* L.). Both produced a mottling disease in white clover (*T. repens* L.), and bright yellow spotting in leaves of the Improved Long Green cucumber (*Cucumis sativus* L.). The similarity in the rather distinctive reactions of the 2 viruses in these hosts strongly suggested that they might represent strains of one virus.

That potato-calico and alfalfa-mosaic viruses are not identical is shown by the fact that the former is the milder of the two in *Nicotiana glutinosa*, crimson clover, and red clover, whereas the latter is the milder in potato. Moreover, under comparable conditions, the potato-calico virus produces fewer lesions in kidney beans, broad beans and cowpeas than does alfalfa-mosaic virus.

CROSS PROTECTION TESTS

Cross-inoculation experiments were made on *Nicotiana glutinosa* and *N. tabacum* L. var. Turkish. It should be pointed out that in *N. glutinosa* both viruses cause diseases showing an acute stage with severe symptoms followed by a chronic stage with mild symptoms. Groups of six young *N. glutinosa* plants were inoculated with one or another of the viruses causing the following diseases: Alfalfa-mosaic, potato-calico, potato-ringspot (caused by *Marmor dubium* H. var. *annulus* H.), cucumber-mosaic (caused by *M. cucumeris* H. var. *vulgare* H.), and Canada-streak. Juice from healthy *N. glutinosa* plants was rubbed over the leaves of six additional plants. Twelve days later, when the inoculated plants were systemically infected, three upper leaves on each of three plants in each group were inoculated with juice from *N. glutinosa* plants infected with alfalfa-mosaic virus. Leaves on the remaining three control plants in each group were similarly rubbed with juice from healthy *N. glutinosa* plants. The plants were observed for two weeks. None of the control plants rubbed with juice from healthy plants developed additional symptoms. Of the leaves rubbed with juice containing alfalfa-mosaic virus, those previously infected by potato-calico virus or alfalfa-mosaic virus were alive and green at the end of this period; the others were dead or moribund. Moreover, the new leaves on the plants previously infected with potato-calico or alfalfa-mosaic showed no symptoms in addition to those characteristic of the chronic stages of these diseases while new leaves on the other plants developed systemic necrotic lesions typical of the acute stage of alfalfa-mosaic.

Similar results were obtained when plants of *Nicotiana tabacum* var. Turkish that had previously been infected with the potato-calico virus were inoculated with alfalfa-mosaic virus. The inoculated leaves of these plants developed no necrotic primary lesions, whereas leaves of previously healthy

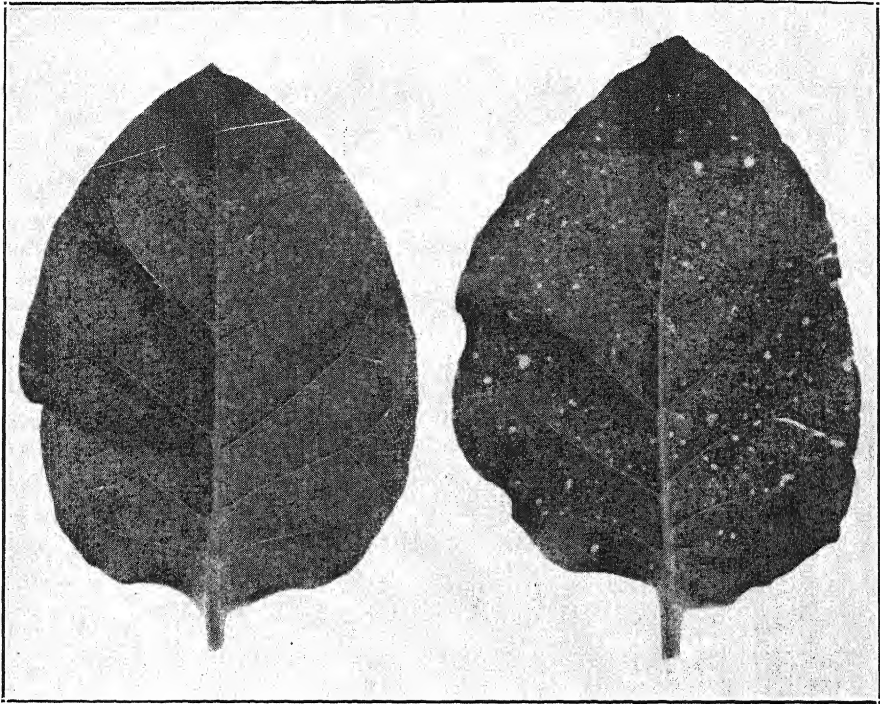


FIG. 1. Leaves from a cross inoculation test on *Nicotiana tabacum* var. Turkish. Both leaves were inoculated with alfalfa-mosaic virus. The leaf on the right had previously been healthy, that on the left had been invaded by the virus of potato calico. The symptoms of potato calico were mild and do not show in the photograph. (Photograph by J. A. Carlile.)

plants (Fig. 1) or plants infected with cucumber-mosaic virus developed many such lesions.

The protection described above is considered good evidence that potato-calico and alfalfa-mosaic viruses are closely related. The potato-calico virus is, therefore, classified as a strain of *Marmor medicaginis* and given the varietal name *solani* from NL. *Solanum*, generic name for the potato. Alfalfa-mosaic virus should be designated as *M. medicaginis* H. var. *typicum* n. var. to distinguish it from the potato-calico strain of the virus.

SUMMARY

Potato-calico virus and alfalfa-mosaic virus produce similar but not identical symptoms on *Nicotiana glutinosa* L., *Phaseolus vulgaris* L., *Vicia faba* L., *Vigna sinensis* Endl., *Solanum tuberosum* L., *Trifolium incarnatum* L., *T. pratense* L., *T. repens* L., and *Cucumis sativus* L.

Plants of *Nicotiana glutinosa* and *N. tabacum* infected with potato-calico virus are refractory to infection with alfalfa-mosaic virus. Potato-calico and alfalfa-mosaic viruses are, therefore, considered to be closely related and the potato-calico strain is named *Marmor medicaginis* H. var. *solani* n. var. Plants affected by potato-ringspot, cucumber-mosaic or Canada-streak are susceptible to infection with alfalfa-mosaic virus. Therefore, the viruses causing these diseases are not thought to be closely related to alfalfa-mosaic virus.

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AN INCUBATING CAN FOR LABORATORY OR FIELD USE

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(Accepted for publication Dec. 4, 1939)

The advantages of glass for many kinds of laboratory receptacles must sometimes be sacrificed for materials of greater durability in work carried on in temporary laboratories or in the field, and initial cost, as well as replacement costs, may also become important. Metal receptacles have not been widely used in place of glass because of the unavailability of a range of sizes and forms, the tendency of ordinary tinned iron vessels to corrode, and the expense of noncorrosive metals. Improvement in lacquering processes is now overcoming some of these difficulties.

In connection with investigations of azalea flower spot (*Ovulinia azaleae* Weiss) carried on in an improvised field laboratory at Magnolia Garden, near Charleston, South Carolina, from 1936 to 1939, we had occasion to keep a large number of flowers under observation for periods of several days up to 2 weeks. It was essential that each sample be segregated to prevent accidental infection, and it was desirable that all samples be kept under approximately uniform conditions of temperature and atmospheric humidity. Celluloid cages were first tried for this purpose in the form of a cylinder about

10 inches high and 5 inches in diameter mounted on a wooden base, which inclosed the neck of a 6- or 8-ounce bottle. For our purpose these proved too bulky, expensive, and difficult to clean and they had the further defect of contributing some toxic emanation to the atmosphere within the cage so that a very low proportion of infection was obtained.

Tin-plated 1-lb. coffee cans were next tried. A 2-dram homeopathic vial was fastened to the inside next the bottom, using either a strip of adhesive tape or a wire passing through the wall with the ends twisted together outside. The vial was buttressed with high-melting-point paraffin so as to form an even slope from its mouth to the bottom of the can and thus eliminate crevices at its base, which would be difficult to clean. In use, the vial was filled with water from a rubber syringe, and an azalea twig bearing 1 to 4, usually 2, flowers was inserted. A piece of paper towel was placed on the bottom of the can to take up any excess water. The flowers were exposed to insect contact or inoculated in various ways, and were usually atomized with water before the cans were covered and set away. Infection by *Ovu-linia* was readily obtained when only a small drop of a spore suspension in water was placed with a pipette on each petal, without atomizing. Such drops usually persisted, without evaporating, for at least 24 hours. Freshly cut azalea flowers remained in good condition in these cans for 8 to 12 days, which was more than ample for the required observations. About 200 of these cans could be stacked in an improvised thermostatic case, approximately 40 inches in each dimension. The consistency and uniformity of infection obtained in replicate samples throughout the case showed that the

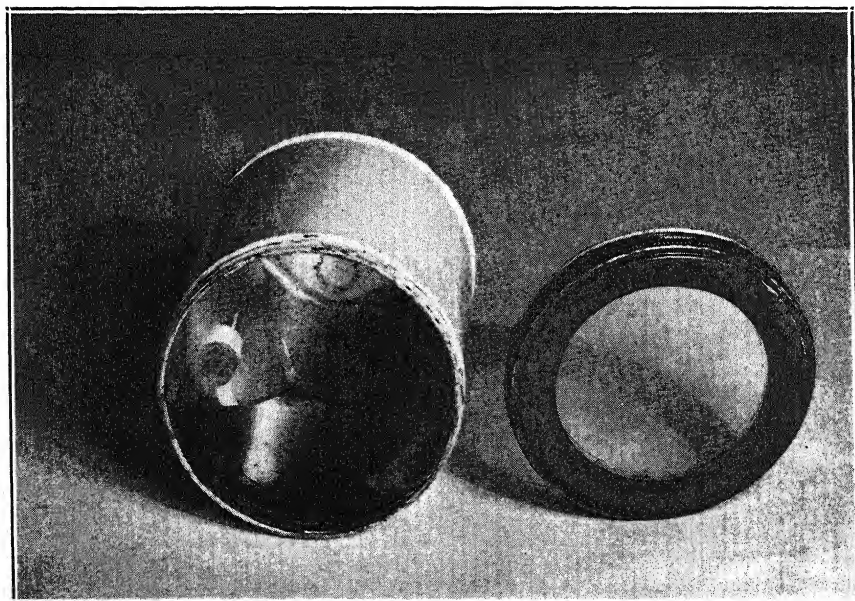


FIG. 1. Incubating can with vial as water reservoir and metal ring and celluloid disc as cover.

requirements of homogeneous moisture and temperature conditions were well met.

An improvement in this type of can was found in a lacquered can intended for the sale of a corn chip or cracker. This can was 4 in. in diameter and $4\frac{1}{2}$ in. high (Fig. 1). The lacquer prevented rusting, and the top, instead of depending on friction, was provided with a grip lock, easily removable yet capable of firm attachment. These cans could be carried, in cartons of 24, into the field on distant collecting trips; they were light, unbreakable, and not easily upset.

These "incubating cans" have been used for experimental inoculations with other fungi, including *Cladosporium paeoniae*, *Puccinia malvacearum*, etc. Small cut shoots and stalked leaves keep in good condition in them up to 2 weeks or longer. Suitable atmospheric humidity for infection seems to be more uniformly maintained in this type of vessel than in the usual moist chamber or bell jar, because of the tight cover and the relatively small surface on which condensation of moisture can occur.

The cans can be satisfactorily cleaned for most purposes by thorough washing with soap and water, but if one wants to dispense with the paraffin buttress around the vial, they may be sterilized in an autoclave or a drying oven. Exposure to 15 pounds of steam pressure did not visibly affect the lacquer, and heating at 243° C. for $2\frac{1}{2}$ hours in a drying oven only darkened it.

For experimental inoculations influenced by light, the top of the can may be cut out on a lathe or with a circular metal cutter to form a ring, and a disc of celluloid inserted between the can and the cover.

The cost of these cans, when obtained from the manufacturer in quantities of 5000, is approximately $4\frac{1}{2}$ cents each, as compared with 25 cents to \$1.00 for Petri dishes and glass "moist chambers."

BUREAUS OF PLANT INDUSTRY, AND OF ENTOMOLOGY AND
PLANT QUARANTINE,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES

Losses from Bunt of Wheat in the United States.—The difficulty of estimating crop losses caused by disease is admittedly so great that there exists some skepticism as to the validity of the published estimates. It is apparent then that any evidence making it possible to check some of these estimates against another method of measuring disease losses, is worth consideration. In figure 1, A, are presented the estimated losses from bunt of wheat in the United States, as compiled from reports from collaborators of the Plant Disease Survey, and the percentage of all ears grading smutty, as indicated by reports of federal grain inspectors. The accuracy of these reports of federal inspection and their usefulness as a source of plant-

disease information have been commented on elsewhere,¹ and Haskell and Boerner² have noted specifically the correlation between the amount of bunt in the field and the smuttiness of threshed grain.

The figures for percentage of all cars grading smutty have been com-

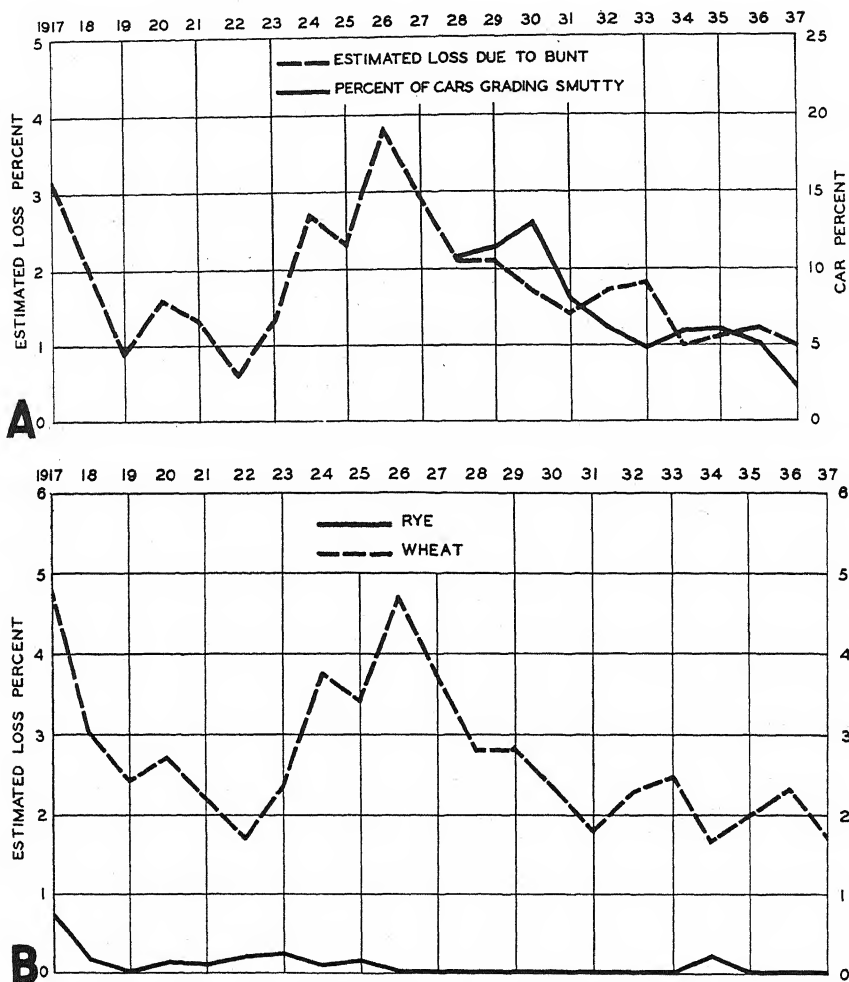


FIG. 1. A. Estimated losses from bunt of wheat in United States (reporting area) 1917-1937 and percentage of cars grading smutty at all terminals, 1928-1937. B. Estimated losses from smuts of wheat and rye in United States (reporting area) 1917-1937, compiled from reports from collaborators of the Plant Disease Survey.

puted for all the different kinds of wheat, beginning with the season of 1928-1929, and were given to the writer by Fred G. Smith, Chairman of the Educational Committee of the Office of Federal Grain Supervision. From

¹ Stevens, N. E. Incidence of ear rots in the 1916-1933 corn crops. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 19: 71-93. 1935.

² Haskell, R. J., and E. G. Boerner. Relation of stinking smut of wheat in the field to smuttiness of threshed grain. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. Sup. 79: 1-5. 1931.

these we have computed the figures for the United States as a whole. When compared with the estimated losses in wheat caused by bunt, it is evident that the two show, in general, the same trend—a decline in the abundance of bunt in the United States during recent years. When it is remembered from what wholly different sources these figures are derived, and that a number of States are not included in the crop-loss estimates, the agreement certainly appears significant.

The same sources of information afford a means of checking the relative smut-induced losses in wheat and in rye. Among the “special grades” the definitions of “smutty” are identical for wheat and for rye,³ and smutty cars of rye are occasionally reported. The number of cars of rye grading smutty during the decade for which figures are available has, however, been too small to be recorded on the graph. This difference in the disease relations of the two crops agrees with the reports of the collaborators of the Plant Disease Survey (Fig. 1, B).

Comparison of A and B of figure 1 shows how large a part of the total estimated loss from smuts in wheat in the United States during this period was due to bunt.—NEIL E. STEVENS, University of Illinois, Urbana, Illinois.

Coniothyrium fuckelii Sacc. on Rose Leaves.—Young leaves of rose, variety Joanna Hill, which had been inoculated with *Diplocarpon rosae* and kept floating on sucrose solution for 7 days, revealed pycnidia of some other fungus within the black-spot lesions. This fungus was identified as *Coniothyrium fuckelii* Sacc. It was isolated, and inoculated into rose canes

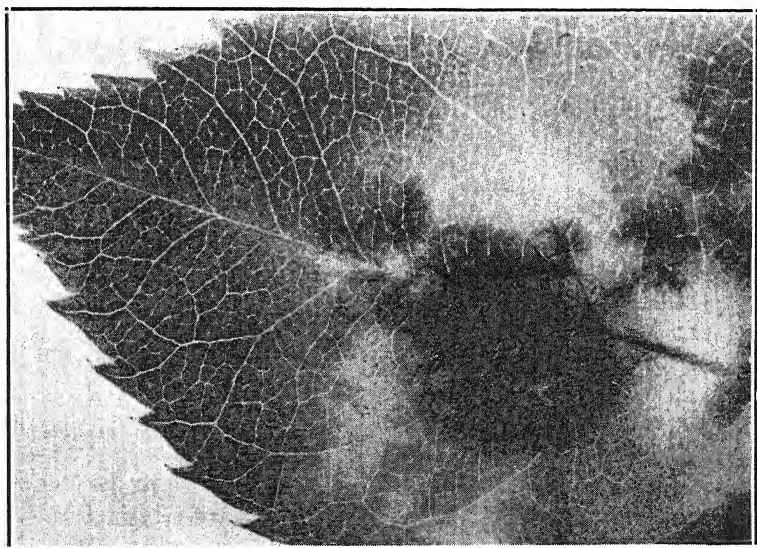


FIG. 1. Pycnidia of *Coniothyrium fuckelii* within black-spot lesion caused by *Diplocarpon rosae*. Photographed two weeks after inoculation. $\times 4\frac{1}{2}$.

³ Handbook of Official Grain Standards of the United States. U.S.G.S.A.—Form No. 90, Revised June, 1937.

where it caused lesions typical for those of the stem and graft canker of roses.

Uninjured young leaves of rose shoots of the varieties Joanna Hill and Talisman, when inoculated with a spore suspension of this fungus and kept for 2 weeks under favorable conditions of temperature and humidity, remained healthy; whereas leaves inoculated with a mixture of spores of *Coniothyrium fuckelii* and *Diplocarpon rosae* bore pycnidia of the former fungus intermingled with acervuli of the latter within the black-spot lesions. Pycnidia were found both on the upper and the under leaf surfaces, and bore numerous spores.

It thus seems that *Coniothyrium fuckelii*, the causal organism of the stem and graft canker of roses, is able to fruit on rose leaves attacked by *Diplocarpon rosae*; whether it is able to follow other pathogens of the rose foliage, has not been determined.

Since close inspection is necessary in order to distinguish the fruit bodies of the two fungi under discussion, the occurrence of *Coniothyrium fuckelii* on black-spotted rose leaves might have escaped attention, although nothing is known yet as to what extent that fungus does follow black spot under greenhouse conditions.—KARLA LONGRÉE, Department of Plant Pathology, College of Agriculture, Cornell University, Ithaca, N. Y.

Heterothallism in Venturia pirina.—The 8 spores of each of 5 asci of *Venturia pirina* Aderhold were isolated in the order of their occurrence in the ascus and grown *in vitro*. Petri dishes containing 20 cc. each of 0.5 per cent malt-extract (Trommer's) agar plus a decoction of dead pear leaves were seeded with conidia from these isolates, used singly and in every possible pairing within each set of 8. Similar seedings were made on sterilized dead pear leaves in test tubes containing a few cc. of malt-extract decoction. The cultures were incubated 4 months at temperatures favorable for the development of perithecia.

All the isolates proved to be hermaphroditic and self-sterile. Each set is comprised of 2 groups of 4 isolates each, which are intra-group sterile and inter-group fertile. Segregation for sexual compatibility occurred alternatively in the first or the second nuclear division in the ascus. Pairings of the isolates between certain sets revealed only 2 compatibility groups.—M. H. LANGFORD and G. W. KEITT, University of Wisconsin, Madison, Wisconsin.

A Preliminary Report on Variability and Inheritance in Venturia inaequalis.—The 8 ascospores of each of 4 asci were isolated in the order of their occurrence in the ascus and grown on malt agar through 12 successive monoconidial transfers made at approximately 8-week intervals. The thalli showed distinctive morphological characters, which were comparatively constant in culture. Each set of 8 isolates contained 4 pairs that behaved alike, indicating that the third nuclear division in the ascus is equational.

The pathogenicity of all cultures was studied 2 seasons on 9 apple varieties. Differential varieties were found for all sets. Pathogenicity, under approximately the same environmental conditions, remained comparatively constant.

A method was developed for growing the ascigerous stage of the fungus *in vitro*. The 32 isolates of the 4 sets fell into 2 groups of 16 each for sexual compatibility, being self-sterile, intra-group sterile, and inter-group fertile. Segregation for sexual compatibility occurred alternatively in the first or second nuclear division in the ascus.

Occasional sectors appeared in culture. They grew faster, produced fewer conidia, and were less pathogenic than their parent isolates. One incited white lesions on leaves. In several cases a sector and its parent isolate were separately paired *in vitro* with the same compatible isolate. The ascocarps from pairings involving the parent isolates had 8-spore asci. Those from 2 pairings involving sectors had 4-spore asci, indicating that these sectors differed from their parent isolates in genetic constitution.—G. W. KEITT and M. H. LANGFORD, University of Wisconsin, Madison, Wisconsin.

A Note on the Status of the Generic Name Urocystis.—In the interest of stability in nomenclature, present trends in mycology and phytopathology are definitely toward adherence to the present International Rules of Botanical Nomenclature. Strict interpretation of these rules, however, would necessitate a number of changes in well-known generic names, established by usage over many years. To obviate undesirable changes of this character provision was made, at the International Botanical Congresses at Cambridge and Amsterdam, for a special committee on fungi authorized to consider the matter of *genera conservanda*. The report of this committee, including a list of generic names proposed for conservation, is to be presented to the next Congress. Even though it is not yet known when the next Congress will convene, it seems desirable to continue the work.

Of the various genera involved, *Urocystis* has been of particular interest, and has been included in the list of proposed *nomina generica conservanda* published by the Nomenclature Committee of the British Mycological Society.¹ The Plant Pathological Committee of the same Society also has endorsed this proposal.² As pointed out in the above reference, the name *Tuburcinia* is the legal name under the provisions of the International Rules, but the following reasons are advanced for conserving *Urocystis* Rabenh. (1856) against *Tuburcinia* (1832).

“(1) The name *Urocystis* has been well known to, and in frequent use by, plant pathologists since there has been a science of plant pathology, and should, accordingly, not be discarded without cogent reasons.

“(2) The disuse of the name *Urocystis* is not dictated by the accession of any new knowledge. It has been known and accepted that *Tuburcinia*

¹ Trans. Brit. Myc. Soc. 23: 223. 1939.

² Loc. cit. 231. 1939.

Orobanches is a species of *Urocystis* since 1877, when it was renamed *Urocystis Orobanches*. For more than forty years mycologists refrained from transferring the species of *Urocystis*, *en bloc*, to *Tubercinia*, so as not to confuse the literature.

“(3) Since 1887, the generic name *Tubercinia* Fr. has been used in a rather different sense, as if it were founded on *T. Trientalis* Berk. and Br., a species unknown to Fries. It is still a matter of taxonomic dispute whether species of *Tubercinia* so used (and none of them are major pathogens) are properly classified in the same genus with the species of the genus *Urocystis*.”

For the above reasons the present committee recommends the continued use of the generic name *Urocystis* until such time as an International Botanical Congress shall have definitely passed on the proposal to conserve it.—Committee on Fungus Nomenclature, G. L. ZUNDEL, *Chairman*, J. A. STEVENSON, C. M. TUCKER, D. S. WELCH, AND ERDMAN WEST.

BOOK REVIEWS

MOORE, W. C. *Diseases of Bulbs*. Bull. 117, Min. Agric. and Fish. Gt. Britain. 176 pp., 58 figs. 1939. Publ. by H. M. Stationery Office, London. Pr. 4 s. U. S. Office, Brit. Libr. Information, 270 Madison Ave., New York. Pr. \$1.20.

This publication should go far to dispel any connotation of mediocrity that often attaches to government agricultural bulletins. It is immediately noteworthy as an example of skilful and artistic presswork, with fine quality of paper and clear type. The 58 half-tone illustrations are superlative in both photographic excellence and clear reproduction. The value of the text for general information and as a reference work is even more appealing to one who is familiar with the subject matter. It covers the diseases of all the major bulbous crops of the Amaryllidaceae, Iridaceae, and Liliaceae and includes those of such relatively unknown (pathologically) bulbous plants as *Colchicum*, *Convallaria*, *Muscari*, *Ornithogalum*, *Scilla*, and others. For the parasitic diseases the treatment includes not only the customary descriptive and historical material, and an authoritative discussion of control, but also a complete mycological account with authentic information on synonymy, morphology, and life history of the causative organisms. The latter feature should especially commend itself to American students of the pathology of ornamentals, since much of the earlier mycological work on bulbs was published in garden and nursery periodicals, which were not adequately indexed in this country. Those written in the Dutch language have remained largely unknown here for want of competent translations. There are 709 literature citations! Practical bulb growers and students will find nothing in the English language to compare with it.—FREEMAN WEISS.

COOK, MELVILLE THURSTON. *Enfermedades de las plantas economicas de las Antillas*. (Translated from the original manuscript by José I. Otero.) Monograph of the University of Puerto Rico (Rio Piedras) Ser. B. (Physical and Biological Sciences), No. 4. 530 pp., frontispiece, 171 figs. 1939. \$2.00.

Although there have been numerous workers in the field of phytopathology throughout that vast area included in Spanish-speaking America, with many and important contributions to the science, no general work on the subject has appeared in the Spanish language for this region until the publication of the present volume.

Pertinent papers have been published quite commonly in the United States or Europe, usually in languages other than Spanish, or they have appeared in local agricultural journals of limited distribution or in more or less ephemeral bulletins and reports that are relatively unavailable to those in need of the information.

The present volume becomes the first Spanish plant pathology to be published in the New World. In point of present-day usefulness it is the only such text available from any source. Gonzalez-Fragoso, it is true, issued his *Botanica Criptogamica Agricola* in 1927, but it is very definitely adapted to the crops and conditions prevailing in Spain, has been difficult to obtain, and is now presumably no longer available. Translations of

the French and Italian texts of Delacroix and Maublanc, and of Ferraris are decidedly obsolete and, of course, were never applicable to new-world conditions to any great extent.

As the title indicates, the work relates primarily to the Antilles; but plant diseases know no political boundaries, so that it is unfortunately true that most of the ills to which the crop plants of the West Indies are heir, likewise occur throughout the tropical and subtropical areas of the New World.

It need hardly be said that the author is particularly well-fitted for the task involved in preparing the book, since he has been actively engaged in the study of plant diseases for many years. Of this time, some 20 years have been spent in Tropical service in Cuba and Puerto Rico of the Greater Antilles. Dr. Cook has been very fortunate in the translator who has collaborated with him, and whose contribution consists of far more than a mere substitution of Spanish words for the English. He has attempted with much success to encompass the spirit of the original and produce a text adequate in all ways to carry out the purpose of the author. In so doing he notes that difficulties were experienced, not the least of which was the lack of technical terms in the language of Castile, which necessitated much extra effort in searching for satisfactory terms.

The author introduces his treatise with a brief history of the subject, outlining it by pertinent references previous to 1900. After a brief account of the physiology of plants, the following 40 pages are devoted to a general discussion of the causes, symptoms, manner of transmission, and the control of plant diseases. The body of the work consists of 442 pages, of which 136, or almost exactly 30 per cent, are devoted to the diseases of sugar cane. This amount of space is not at all out of proportion to the importance of this crop in the Antilles. *Saccharum* and its many diseases have been studied intensively over a long period of years, almost to the exclusion at times of other crops. This condition is reflected in the present work, which summarizes adequately the present state of knowledge of 31 diseases recognized for Cuba and Puerto Rico and gives brief accounts of various other parasitic and nonparasitic diseases reported for other cane-growing countries.

Second in rank are the citrus diseases with 65 pages, followed in order of importance by the banana with 38, cacao with 26, coffee with 15, tobacco with 12, pineapples with 10, the vegetables as a group with 90, minor fruits and other economic plants accounting for the balance. An effort appears to have been made to deal in so far as space limitations permitted not only with the many diseases actually present in the Antilles, but to record those that occur in other parts of Tropical or sub-Tropical America. Some important Oriental diseases, such as the dread coffee rust, also are discussed. Both the author and the translator have been interested in virus diseases, as is evidenced by their published bibliographies on the subject, and the viruses have not been overlooked, particularly in the case of sugar cane, which has most certainly had more than its share of this type of disease.

Diseases due to unfavorable environmental conditions, to nutritional deficiencies, and other parasitic causes are adequately treated. The section devoted to vegetable diseases is of particular interest, since the growing of various of these crops is a comparatively new development, both for home use and export, and the diseases have been much in evidence as efforts have been made to expand vegetable production.

As a pioneer in its field, it is to be expected that errors have crept in, which a later edition can remedy. An unfortunately large number of typographical errors will be found. There are 5 on page 327, for instance. The author has not been consistent in capitalizing specific names in following the International Rules nor in abbreviating authorities for technical names. He has not clearly differentiated between fungi directly parasitic in plants and those attacking insect pests of the plants treated. Similarly *Zygosporium oschiodes* is a parasite of *Pucciniopsis* on *Carica* and not directly of *Carica* itself (p. 321). Onion smut (p. 387) is attributed to *Uromyces* rather than to *Ustilago*. Most workers would take issue with the statement that *Stilbella flavida* can be combatted easily "facilmente," with Bordeaux Mixture.

A very disappointing feature is the fact that many of the illustrations have been so poorly reproduced as to be worthless, for which, however, we cannot blame the author.

A bibliography has not been included in the interest of space conservation, and the reader is referred to the author's and translator's recently issued "Bibliography of Mycology and Phytopathology of Central and South America" published in *The Journal of Agriculture of the University of Puerto Rico* 21: 249-486. 1937. This bibliography is said to be still available from the compilers at the Experiment Station, Rio Piedras, Puerto Rico.

In view of the difficulties encountered by the translator in finding adequate Spanish technical terms and the resulting need for developing a satisfactory Spanish terminology for the sciences, it has been a most commendable plan to include a glossary, which, briefly but adequately, defines each term used in the text and gives an English equivalent.

The book is completely and apparently carefully indexed as to scientific names of parasites and the common names of diseases and hosts. It does not include the scientific

names of the hosts, however, so that the reader, unfamiliar with Spanish common names or with the particular common name used, since these vary greatly from country to country, may experience difficulties in locating a given crop plant.

The work is distinctly timely and will fill a definite need on the part not only of the actual workers in the specialized field, but of those engaged in extension work, an activity now coming into its own in Puerto Rico, and other Latin-American countries, of agriculturists in general, and of all who are in any way concerned with the problems presented by disease in plants.—JOHN A. STEVENSON, Bureau of Plant Industry, Washington, D. C.

HOLMES, FRANCIS O. *Handbook of Phytopathogenic Viruses*. 221 pp. Price \$2.00. Burgess Publishing Company, Minneapolis, Minn.

This handbook of plant viruses is a very much enlarged edition of the author's paper on a "Proposal for extension of the binomial system of nomenclature to include viruses" (Phytopath. 29: 431-436, 1939) in which he proposes a latinized binomial system of nomenclature for plant viruses. The present volume includes a classification and nomenclature of only the better-known plant viruses.

The table of contents gives in outline form the author's earlier attempt to classify and name the plant viruses. In the text the viruses that affect higher plants and bacteria are classified under kingdom, divisions, classes, families, genera, species, and varieties. The basis of classification is nearly entirely symptoms, rather than any fundamental characters of the viruses themselves. The family Chlorogenaceae and genus *Chlorogenus* includes the viruses of the yellows diseases, as aster yellows, peach yellows, little peach, potato witches broom, etc., all of which are leaf-hopper transmitted; but it does not include all leaf-hopper transmitted viruses. The family Marmoraceae, genus *Marmor*, includes viruses causing mottling or necrotic spotting of the host; and Annuaceae the viruses causing ring patterns, with "recovery" and eventual "non-sterile immunity." The formation of vascular proliferations or galls is the basis for including other viruses in the family Gallaceae. Spindle tuber, leaf curl, leaf roll, dwarf disease, savory disease, and spotted-wilt families complete the viruses attacking the higher plants.

Each species or variety of the better-recognized viruses is described in detail giving Latin and common names and some synonyms, a partial list of susceptible and sometimes insusceptible species, distribution, induced diseases, transmission, serological, and "immunological" relationships, properties, and control, with a selected list of literature. These descriptions of the viruses should prove valuable for reference purposes. In supplement I, 28 pages are devoted to Bacteriophages of animal and plant bacteria, giving much the same type of information for each species of the genus *Phagus* as is given for the plant viruses. Supplement II lists the hosts for some of the better-known viruses, as aster yellows, curly top, cucumber mosaic, etc., and lists both the hosts and species nonsusceptible to the tobacco mosaic virus. Supplement III lists viruses that for one reason or another are not treated in the handbook. An index follows, in which the page numbers of viruses fully described are underlined. The material throughout appears to be well selected and is put in an orderly, concise form, so that information is readily accessible.

The publication of this book obviously adds to the confusion already present in plant-virus nomenclature, and, one might add, to classification also. Perhaps this is desirable until some system of classification and nomenclature is finally adopted; as one would hesitate when writing about the tobacco-mosaic virus to refer to it as Tobacco virus 1, Johnson; Nicotiana virus 1, Smith; *Marmor tabaci* var. *vulgare* Holmes, or common tobacco-mosaic virus; but may be content to refer to it as the tobacco-mosaic virus. If the latter course is followed for the present no harm will have been done in placing before those interested in viruses another proposal for naming them, and those interested in classification will have another basis for grouping to consider.

It does not seem that full advantage has been taken of our present knowledge of the viruses in classifying them. For example, the genus *Marmor* includes the tobacco-mosaic virus, the serologically related English cucumber-mosaic virus, the unrelated American cucumber-mosaic virus, the etch virus, and many others that cause mottle symptoms in their respective hosts, and all are coordinate without regard to relationship. It would seem that tobacco-mosaic virus, American cucumber-mosaic virus, etch virus, potato ring-spot virus, and perhaps some other viruses in the genus *Marmor* are distinct kinds and should be given generic rank. Then the English cucumber-mosaic virus and tobacco-mosaic virus could be considered species of a common genus. The vein-banding virus is improperly treated as a strain of the cucumber-mosaic virus. If future studies confirm the claim that it reacts serologically with antigens of cucumber mosaic virus it cannot even then be considered coordinate with a mutant strain of the cucumber virus, but should be considered a distinct species. It should be kept in mind that cucumber-mosaic

virus and vein-banding virus are frequently found associated in the same tobacco plant and that one gives no protection against the other.

The author, possibly without recognizing it, is laying the foundation for endless trouble, if his proposal to name strains of constantly mutating viruses is followed. The laboratory workers may feel justified in naming mutant strains, but to the person working with the field strains the naming of strains of the constantly mutating viruses becomes unthinkable. *Marmor tabaci* var. *vulgare* is the name given to the "typical strain" of the tobacco mosaic virus, with tobacco virus 1 and *Nicotiana* virus 1 as synonyms. By definition these latter terms mean the common field mosaics that have been recognized the past 50 years. Either the author believes that there is only one common field strain, which he names var. *vulgare*, or, if he recognizes the true situation, namely that there are numerous distinct field strains of the common tobacco-mosaic virus, he is willing to classify all of them under one varietal name. The tobacco ringspot and the cucumber-mosaic viruses are also made up of numerous strains in nature and it is questionable whether any one strain of the cucumber-mosaic virus is common enough to be called var. *vulgare*. Recognition of endless variations or mutations of viruses, such as the tobacco-mosaic virus, both in the field and laboratory, is essential, but to name each mutant with other than a laboratory designation will result only in confusion.

Where strains of a virus are well established in nature and cause a well-recognized disease year after year, the use of a varietal name seems justified. For example the little-peach virus is named *Chlorogenus persicae* var. *micropersica*. It is questionable, however, whether pathologists will submit to using *Chlorogenus persicae* var. *vulgaris* for the peach yellows virus when *Chlorogenus persicae* will do.

The use of the term "immunity" throughout the text, in referring to failure of a virus to cause a repetition of symptoms in a plant already affected with a slightly different strain of that virus, is unjustified. The plant is not immune from either virus, but is "protected" to a greater or less degree by the first virus against the second.

The reviewer hesitates to express an opinion regarding the advisability of adopting a latinized system of nomenclature for viruses, but it is such a marked advance over any proposal yet offered that it should be given very careful consideration.

Aside from what one may think of the advisability of applying a binomial system of nomenclature to viruses at this time, the book probably will prove a welcome addition to the virus literature, as it gives in a small, compact, loose-leaf type of book a list of viruses of plants and, concisely and apparently accurately, what is known of them.—W. D. VALLEAU, University of Kentucky, Lexington, Ky.

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

1940 SUMMER MEETING

SEATTLE, WASHINGTON—JUNE 19-22

In conjunction with the Program of the American Association for the Advancement of Science and Affiliated Societies, June 17-22.

Wednesday, 9:00 a.m.—Business meeting of Pacific Division of The American Phytopathological Society.

Wednesday, 10:00 a.m.—Presentation of papers.

Wednesday, 1:30 p.m.—Presentation of papers.

Thursday, 9:30 a.m.—Joint session with Northwest Association of Horticulturists, Entomologists, and Plant Pathologists; Economic Entomologists and Society for Horticultural Science.

Invitation papers dealing with field plot arrangement, statistical analysis, virus vectors, virus physiology, or related topics.

Thursday, 1:30 p.m.—Presentation of papers.

Friday, 9:30 a.m.—Symposium on fruit tree virois diseases.

Saturday: Field trip to Western Washington Experiment Station, Puyallup, Washington. Joint program with Horticulturists and Entomologists. Demonstrations of spray equipment and experimental plots.

Pathologists who wish to present papers at these meetings should submit titles to L. D. Leach, Secretary of the Pacific Division of the The American Phytopathological Society, University of California, Davis, California.

CHAS. CHUPP, <i>President</i>	R. S. KIRBY, <i>Secretary</i>	B. F. DANA, <i>President</i>
Cornell University	Pennsylvania State	Pacific Division
Ithaca, N. Y.	College	U. S. D. A.
	State College, Pa.	Corvallis, Oregon

SUMMER MEETING OF THE NORTH CENTRAL STATES GROUP OF PHYTOPATHOLOGISTS

The North Central States group of phytopathologists will conduct a summer tour in western Illinois from June 20 to 22. The group will assemble at Quincy, Illinois, on June 19. June 20 will be spent on tree fruit and small fruit diseases near Quincy and on grain diseases in the Illinois River bottom near Jacksonville. The afternoon will be devoted to an inspection of the experimental orchard spraying work at Jerseyville. The night will be spent at the famous Pere Marquette State Park near Grafton.

On June 21, the group will tour the intensive vegetable area in the Mississippi River bottom near East St. Louis where various vegetable and field crop diseases will be seen.

Members of the Society other than those in the North Central States (Michigan, Wisconsin, Minnesota, Iowa, Nebraska, Missouri, Illinois, Indiana and Ohio) who plan to attend the meeting should write Dr. H. W. Anderson for detailed program about May 20.

Committee on Arrangements,

C. M. TUCKER

I. H. MELHUS

H. W. ANDERSON

STUDIES ON THE BIOLOGY OF VALSA SORDIDA AND CYTOSPORA CHRYSOSPERMA¹

CLYDE M. CHRISTENSEN²

(Accepted for publication December 1, 1939)

INTRODUCTION

The fungi discussed in this paper are among the most common bark-inhabitating fungi found on our native forest poplars and on the introduced species and varieties of ornamental poplars. They have been considered to be of rather minor importance on our native forest trees, but have been thought to be one of the chief reasons for the poor survival and short life of ornamental poplars in this and some other regions. Despite their prevalence and their at least supposed practical importance, comparatively little is known of some phases of their life. The present studies were undertaken to find out certain facts about the growth, reproduction, and parasitism of the fungi concerned and to determine their range of variation.

SOURCE OF MATERIAL

The sources of the fungi studied are shown in table 1. Unless otherwise stated, the cultures were obtained by suspending masses of spores in sterile water and placing drops of this spore suspension on malt agar slants in test tubes. Special studies were made with single-spore isolates from some collections of the fungi, but, for most of the work, mass spore cultures were used.

TAXONOMY OF THE GENUS VALSA

The taxonomy of this genus has been reviewed in detail by Schreiner (9), and will be considered here only as it applies to the specific problems in this study. Rabenhorst (7) describes 129 species of *Valsa*, 6 of them on *Populus*, and 11 on willows. Some of these several species he considers as collective. Saccardo (8) lists 133 species of *Cytospora*, 3 of which occur on poplar. He limits *Cytospora chrysosperma* to species of poplar and, more recently, Grove (1) has done the same. Hubert (2) reported this same fungus on 12 species of trees, in 6 different genera, and this host range has been extended by other authors. Neither Grove nor Hubert states his reasons for considering the species as he does, the former listing no characters by which the species could be distinguished with certainty from all other species of *Cytospora*; and the latter making only a categorical statement of the identity of the fungus. Obviously there are at present no very positive means of identifying *C. chrysosperma*. If the limits of variation of this fungus on species of *Populus* were established, one would have a basis on

¹ Paper No. 1751 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

² This paper is a summary of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Doctor of Philosophy degree.

TABLE 1.—Source of isolates of *Cytospora chrysosperma* and *Valsa sordida*

Isolate No.	Host	Location in Minnesota	Remarks
<i>Cytospora chrysosperma</i>			
1	<i>Populus tremuloides</i>	University Farm	Living tree.
2	do do	Afton	Isolated from wood near an insect tunnel.
3	do do	Itasca Park	Isolated from wood near Monochamus tunnel in interior of log cut the previous year.
4	<i>P. candicans</i>	University Farm	Dead twigs of living tree.
5	<i>P. alba</i> , var. <i>pyramidalis</i>	Lake City	do
7	do do	Minneapolis	do
8	<i>P. spp.</i> (Russian poplar)	Cloquet	Canker on living tree.
9	<i>Juglans spp.</i> (Japanese walnut)	University Farm	Dead twig of living tree.
12	<i>P. alba</i>	Minneapolis	do
14	<i>P. alba</i> var. <i>nivea</i>	St. Paul	Canker on living tree.
17	<i>P. spp.</i>	Savage	Dead branch of living tree.
19	<i>P. spp.</i> (exotic hybrid)	Excelsior	do
20	<i>P. nigra</i>	Minneapolis	do
23	<i>Ulmus americana</i>	Unknown	Tissue culture from dead branch.
26	<i>P. nigra</i>	Brainerd	Dead branch of living tree.
28	do	St. Paul	do
<i>Valsa sordida</i>			
32	<i>Populus tremuloides</i>	Anoka	Dead tree.
33	do	Wyoming	Dead branch of living tree.
37	do	Unknown	do
39	do	Anoka	do
40	do	do	do
41	do	do	do
42	do	do	do
43	do	do	do
44	do	do	do
46	do	do	do

which collections of the fungus from other hosts could be compared. To establish such a basis it has been necessary to study in considerable detail a number of different characters of the fungi concerned, to find out which of those characters are sufficiently constant and characteristic to be of diagnostic value, and to determine their range of variability. It should thus be possible eventually to make some order out of the taxonomic chaos in which these fungi are involved. The elucidation of the relationship between *C. chrysosperma* and *V. sordida* is a necessary part of this work.

Valsa sordida

Development and Structure of Pycnidia. The pycnidium begins as a clump of densely interwoven hyphae that at first is covered by a layer of bark from 6 to 20 cells deep. At about the time the tip of the pycnidium emerges from the bark, a cavity begins to form in the lower central part of the interior of the stroma. Usually, if indeed not invariably, only one

cavity is initiated. This continues to enlarge in a very irregular manner, so that eventually a cavity is formed that contains many interconnected chambers of various shapes and sizes. There is a wide variation in the shape of these chambers, and 2 pycnidia, growing side by side on a piece of bark inoculated with a pure culture, may differ considerably. The outer wall comprises several layers of pseudoparenchymatous cells. Viewed from the outside the mature pycnidium is irregular in shape, the convolutions of the wall conforming roughly to the shape of the larger chambers within.

Conidiophores arise from the inner layer of cells that form the wall of the chamber. These are filiform, colorless, usually unbranched, but occasionally branched once, and sometimes twice. They are from 10 to 18 μ long and about 1 μ in diameter. Spores are produced at the ends of the conidiophores. A spore first appears as a terminal swelling; this grows until the typical spore length is attained. The conidiophore becomes constricted at the base of the spore, and this constriction increases until the spore is cut off. Although the continued production of conidia by a single conidiophore has not been observed, it seems obvious, from the quantity of spores produced, that each sporophore continues to function for some time.

Development of Pycnidia on Sterilized Twigs. Most isolates, growing on sterilized twigs in flasks, form fewer but larger pycnidia than when growing on the bark of trees in the field. If the bark is not too moist the pycnidia develop normally within the bark; if excess water be present they may form on the surface of the bark, much as they do on the surface of an agar medium. Spores are exuded from some of these pycnidia in coiled yellow spore horns. In these flasks, even when kept in a laboratory where the temperature fluctuates 5° C. or more, there can be comparatively little change in relative humidity. Thus it seems likely that in this case the spores are exuded not so much as a result of swelling of the gelatinous matrix, but rather because the spores and matrix are produced continuously and in such quantity that they are forced out. When the tips of mature pycnidia are cut off a tendril of spores oozes out at once, and the speed with which it appears indicates that there is some pressure within the pycnidium. The prolificacy of the fungus is amazing. On a small twig having a surface area of approximately 30 sq. cm. more than 25 billion spores were exuded in about a week, when the cultures were between 20 and 28 days old, and probably half that number remained within the pycnidia.

Freehand sections were cut transversely through a number of pycnidia of each ascospore isolate. No constant morphological differences were found between the pycnidia of the different isolates, and the variation between different pycnidia of one isolate seemed just as great as between pycnidia of different isolates. A diagram of a typical one is shown in figures 1, B and C.

Size of Conidia. Fifty conidia of each of 5 ascospore isolates of *Valsa*

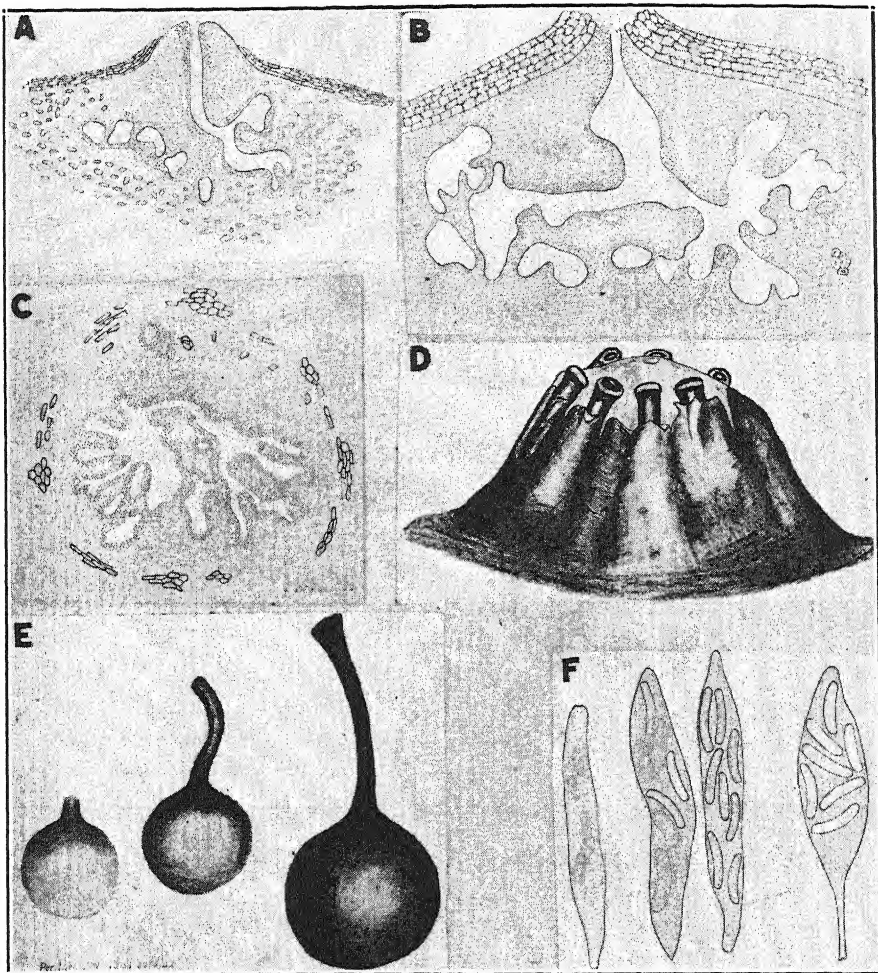


FIG. 1. A. Vertical section through the center of pycnidium of *Cytospora chrysosperma*. B and C. Vertical and cross sections, respectively, through pycnidia of *Valsa sordida*. D. A typical group of perithecia of *Valsa sordida*, the necks coming up around the old pycnidium. E. Perithecia in different stages of development. F. Asci and ascospores, immature on the left, mature on the right.

sordida were measured. The spores were taken from pycnidia produced on malt-agar slants in test tubes, kept in the laboratory. To avoid the possibility, however remote, of getting immature spores, only those spores were taken that had been exuded from the pycnidia. They were suspended in water, a drop of the suspension was placed on a slide, a small drop of warm agar added, and a cover glass applied quickly and pressed down firmly. This method was used because the apparent size of spores was altered significantly by mounting them in some other solid media, in lactophenol, or even staining them with cotton blue. It was found necessary to use some comparatively solid medium, to prevent flowing and Brownian movement, if large numbers of spores were to be measured

accurately. The diluted agar mount did not alter perceptibly the apparent length of the spores. They were measured with a screw micrometer, using an oil immersion lens (Table 2). There is detectable very little difference in length of spores from the different isolates.

TABLE 2.—Length of conidia of different isolates of *Valsa sordida* on *Populus tremuloides* and *Cytospora chrysosperma* on other species of *Populus*

Isolate	Host	Average length of 50 spores, in microns
	<i>Valsa sordida</i>	
40	<i>Populus tremuloides</i>	4.49
41	Do	4.52
42	Do	4.62
44	Do	4.49
46	Do	4.62
	<i>Cytospora chrysosperma</i>	
4	<i>P. candicans</i>	5.73
7	<i>P. alba</i> , var. <i>pyramidalis</i>	5.64
17	<i>P. sp.</i> (probably <i>P. alba</i>)	4.44
19	<i>P. sp.</i> (exotic hybrid)	3.90
26	<i>P. nigra</i> , var. <i>italica</i>	4.06

Structure and Development of Perithecia. Pycnidia, borne on trees in the field, cease to function after a relatively short but very productive life, and the walls disintegrate slowly. Perithecia arise below and in a circle around the old pycnidia. From 1 to 20 perithecia may arise around a pycnidium, the typical number in the material studied being 6 to 8. They arise among the bark cells, and are not surrounded by any definite stromatic tissue, merely sitting loosely in the disintegrated bark. A sketch of the perithecia is shown in figure 1, D and E. The enlargement at the top of the neck of the mature perithecium is typical; frequently adjacent perithecia in a group are grown together at this enlarged portion and may be lifted out of the bark together.

Asci arise as outgrowths from pseudoparenchymatous cells composing the inner wall of the perithecium. All the spores in an ascus are approximately the same size, and the variation between spores in different asci is far greater than that between spores within an ascus. There are typically 8 spores in an ascus, occasionally 7, and rarely 6. It has not been noticed that any of the spores, when fewer than 8 are present, are abnormally large. They usually are arranged in a biserial fashion in the ascus, but this varies a good deal, and often spores are irregularly distributed in the ascus. The spores appear larger when they are first delimited than when they are mature, and the mature ones are quite clear.

The ascus wall is surrounded at all times during its growth by a rather thick gelatinous sheath, which becomes invisible, if present, in many mature asci. Typical asci are shown in figure 1, F. When the ascus is mature it is liberated from the wall of the perithecium. The spores may be lib-

erated in any one of three different ways: 1. Some asci break within the perithecium, and the spores escape, much as do the conidia from the pycnidium, by oozing out, except that the matrix in the perithecia is less gelatinous and less abundant than that produced by the pycnidia. If a mature or over-mature perithecium be broken or cut open, a large number of free ascospores are found within, although very old perithecia may be completely empty. The spores exuded from a fresh perithecium collect around the ostiole in sticky, white masses. 2. Some asci are forced up the neck to the ostiole, where they burst, discharging the spores forcibly into the air. Pieces of bark, collected in March, and bearing mature perithecia of *Valsa sordida*, were placed on moist cotton in a Petri dish, arranged so that the ostioles of the perithecia were about 5 mm. from the cover of the dish. After about 24 hours a deposit of spores, visible to the naked eye, appeared on the glass above one group of perithecia. The spores discharged from each perithecium were in a separate clump. 3. Many of the asci that travel up the neck of the perithecium do not project the spores into the air when they burst, and these collect around the ostiole of the perithecium. In the material observed many more spores collected around the ostioles than were shot out into the air, but the manner of liberation must depend considerably upon the amount of water present.

The perithecial stage has been found by the writer only on *Populus tremuloides*. Perithecia were found on 16 of 30 specimens of *P. tremuloides* collected near Anoka, Minnesota. Perithecia also were found in abundance on specimens collected at the University Farm, Carlton, Wyoming, and Sandstone, which indicates that it probably is formed commonly through central and northern Minnesota, and is not so rare as has previously been stated.

Size of Ascospores. One hundred ascospores from perithecia on each of 5 different specimens collected in an area less than 100 yds. square, near Anoka, were measured with the aid of a screw micrometer. The spores from each specimen were taken from the perithecia around one pycnidium. The perithecia were crushed in a drop of water to obtain a suspension of spores, a small quantity of this spore suspension was placed on a slide, some warm liquid agar added, and a cover glass applied and quickly pressed down. The average length of 100 spores from each of the 5 specimens was 8.2, 8.3, 8.9, 9.9, and 10.4 microns, respectively. The minimum significant difference between any two averages was $0.7\ \mu$. The spores from each specimen were remarkably constant in length, as determined by comparing the average length of the first 50 measured with the average length of the second 50 measured. For example, in collection 45 there was only $0.5\ \mu$ difference, and in collection 34, $0.1\ \mu$ difference between these two averages. Using twice the standard error of the difference as a criterion of the minimum level of significance, there was a significant difference in length between the spores from some of the different collections. Naturally, the cause of this difference is not known, and it cannot be assumed arbi-

trarily that the differences are inherent and that these collections constitute different races. Judging from the above results and from the figures given by other workers, the ascospores of *Valsa sordida* vary considerably in size. If spore length is to be used as one of the criteria of species of *Valsa*, as it has been, this variability obviously must be taken into account.

Cultural Characters. These fungi were cultured chiefly to find out if *Valsa sordida*, from *Populus tremuloides*, could be distinguished from *Cytospora chrysosperma* from other species of *Populus*. The isolates were grown first in test tubes, then repeatedly transferred to detect the presence of other organisms. The first transfer usually was made by taking freshly exuded spores from a pycnidium, suspending the spores in sterile water, and placing a drop of this spore suspension on a malt-agar slant. In most cases this technic insured cultural purity. So far as the writer was able to see, there was no consistent difference in cultural characters between the isolates of *V. sordida*, from aspen, and the isolates of *C. chrysosperma* from other species of poplar. Photographs of cultures of several isolates of *V. sordida* are shown in figure 2, A.

RELATIONSHIP OF CYTOSPORA CHRYSOSPERMA AND VALSA SORDIDA

Numerous authors have followed Nitschke (5) in stating that *Cytospora chrysosperma* is the imperfect stage of *Valsa sordida*. The writer has not found any statement that *V. sordida* has been obtained from *C. chrysosperma*, which should be done to have positive proof of the identity of the fungi concerned. Naturally, it is of some importance to know whether the *Cytosporas* commonly found on ornamental poplars actually are identical with the *Valsa* and *Cytospora* on aspen, or whether two or more fungi are concerned.

Seymour (10), in his host index of the fungi of North America, lists *Valsa sordida* on *Populus tremuloides* and *P. angustifolia*. In the mycological herbarium in the Department of Plant Pathology and Botany, University of Minnesota, the writer examined the following specimens of *V. sordida*: 1. On *Populus* spp. from Decorah, Iowa, collected by E. W. Holway; 2. On *P. pyramidalis*, from Treplitz, Bohemia, Austria, collected by de Thümen. There are 3 pieces of bark in this collection. One bears pycnidia of *V. nivea*, another bears pycnidia, apparently of *V. sordida*, and there are no visible fruiting bodies of any fungus on the third; 3. On *P. nigra*, from Königstein, Germany, collected by W. Krieger. There are 2 specimens, 1 bearing pycnidia, apparently of *V. sordida*, and the other pycnidia and perithecia of *V. sordida*; 4. On *P. tremulae*, from Russia, collected by Ligit Serebriannikov. Only one specimen is present and this bears perithecia of what appears to be *V. nivea*. 5. On *P. nigra*, from France, collected by F. Fautrey. This specimen bears perithecia of *V. sordida*. The writer has found the perfect stage only on *P. tremuloides*.

There are several possibilities to account for the fact that perithecia of *Valsa sordida* are found so frequently on *Populus tremuloides*, and infre-

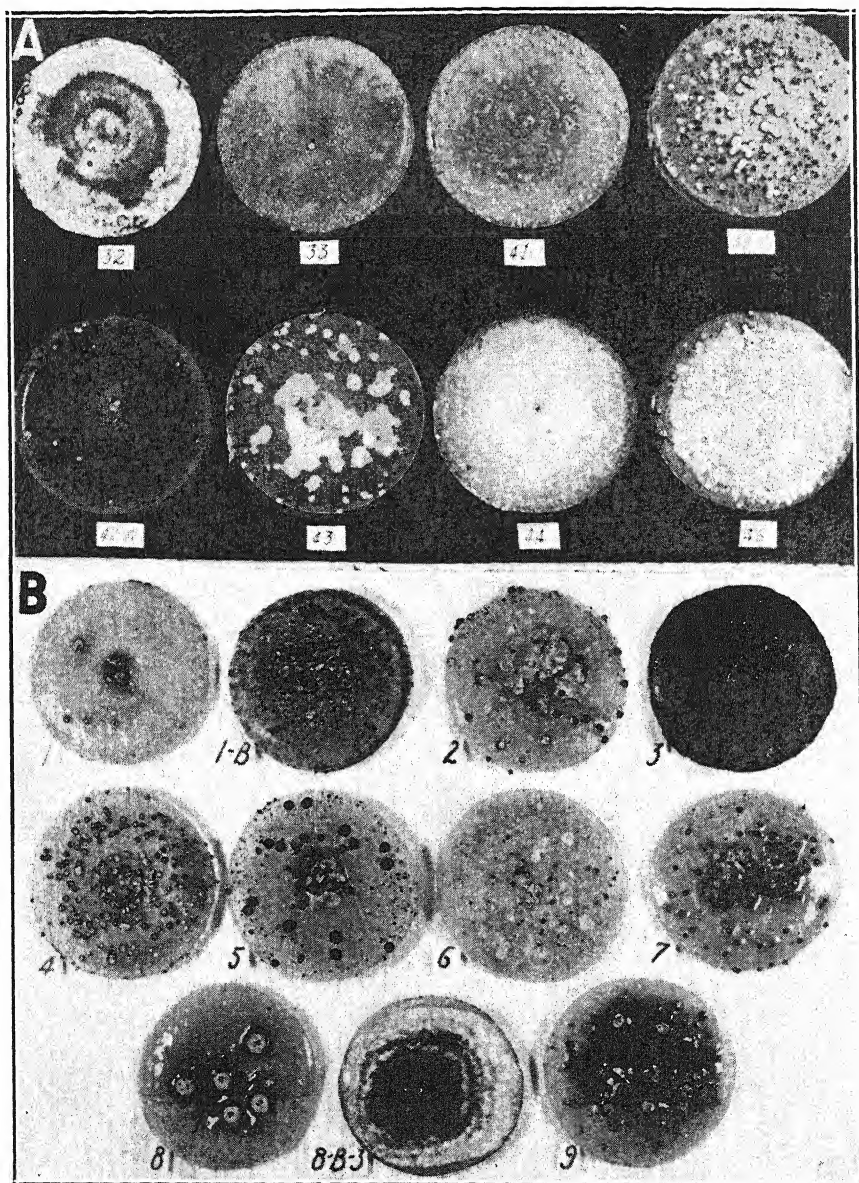


FIG. 2. A. Isolates of *Valsa sordida*, from *Populus tremuloides*. B. Isolates of *Cytospora chrysosperma*: 1, *Populus tremuloides*, Univ. Farm; 1-B, a variant of 1; 2, *P. tremuloides*, Afton; 3, *P. tremuloides*, Itasca Park; 4, *P. candicans*, Univ. Farm; 5, *P. alba* var. *pyramidalis*, Lake City; 6, *Salix discolor*, Itasca Park; 7, *P. alba*, Minneapolis; 8, Russian poplar, Cloquet; 8-B-3, a variant of 8; and 9, Japanese walnut, Univ. Farm.

quently on the bark of other species. 1. *Cytospora chrysosperma* and *V. sordida* are identical, but perithecia are produced only under certain environmental conditions that rarely are encountered on some species and varieties of poplar. 2. There are different strains of the fungus, some of

which are more common on aspen, and readily produce perithecia, while others are more common on other species of poplars and rarely or never produce perithecia. 3. The fungi are taxonomically distinct, although the pycnidia are similar. The writer attempted to settle this problem by trying to induce the isolates of *V. sordida* and *C. chrysosperma* to form perithecia, which would be the only positive proof, by studying the development and morphology of the pycnidia and asexual spores, and by comparing the cultural characters.

Attempts to Induce the Formation of Perithecia. Several isolates of *Cytospora chrysosperma* and *Valsa sordida* were inoculated onto sterile aspen bark, and the resulting cultures were subjected to alternate freezing and thawing and were alternately dried and moistened. Some were kept for over 2 years. A few were exposed to ultraviolet light. Other organisms that commonly grow in old aspen bark were added to some of these cultures. No perithecia were formed on any of the bark cultures. Several isolates were grown on the medium described by Leonian (3) as favorable to the production of perithecia of *V. leucostoma*, but none of the writer's isolates formed perithecia on this medium.

COMPARISON OF THE STRUCTURE OF PYCNIDIA OF CYTOSPORA CHRYSPERMA AND VALSA SORDIDA

Pieces of bark from several different collections of *C. chrysosperma* from species of *Populus* other than *P. tremuloides* were embedded in paraffin, sectioned with the microtome, and examined microscopically. A diagram of a section through the center of one is shown in figure 1, A. No consistent difference could be found between these pycnidia and those of *V. sordida* on aspen.

Seven isolates of *C. chrysosperma*, from several species of *Populus*, were grown on sterile bark in Erlenmeyer flasks, and freehand sections of numerous pycnidia of each isolate were compared with those cut from pycnidia of *V. sordida* grown in the same way. No consistent difference was observed.

Length of Conidia of Cytospora chrysosperma. Fifty spores of each of 5 isolates of *C. chrysosperma* were measured, using the same technic as that previously described in measuring the spores of *Valsa sordida*. The results are given in table 2. The range in size is greater than was found for *V. sordida*, but the conidia of the latter fungus cannot be separated from those of *C. chrysosperma* as a group on the basis of size.

Growth of Cytospora chrysosperma on Sterile Twigs. Several isolates of *C. chrysosperma*, inoculated into the bark of logs kept in a large moist chamber, produced a very abundant crop of pycnidia within 2 weeks. These were slightly larger than those produced on trees in the field, and some of them exuded long tendrils of spores. The spores in the tendril illustrated in figure 3, C were suspended in water and sufficient samples of the suspension counted in a Spencer counting chamber to permit at least a reasonably accurate estimate of the number of spores present. This one tendril contained about 580,000,000 conidia.

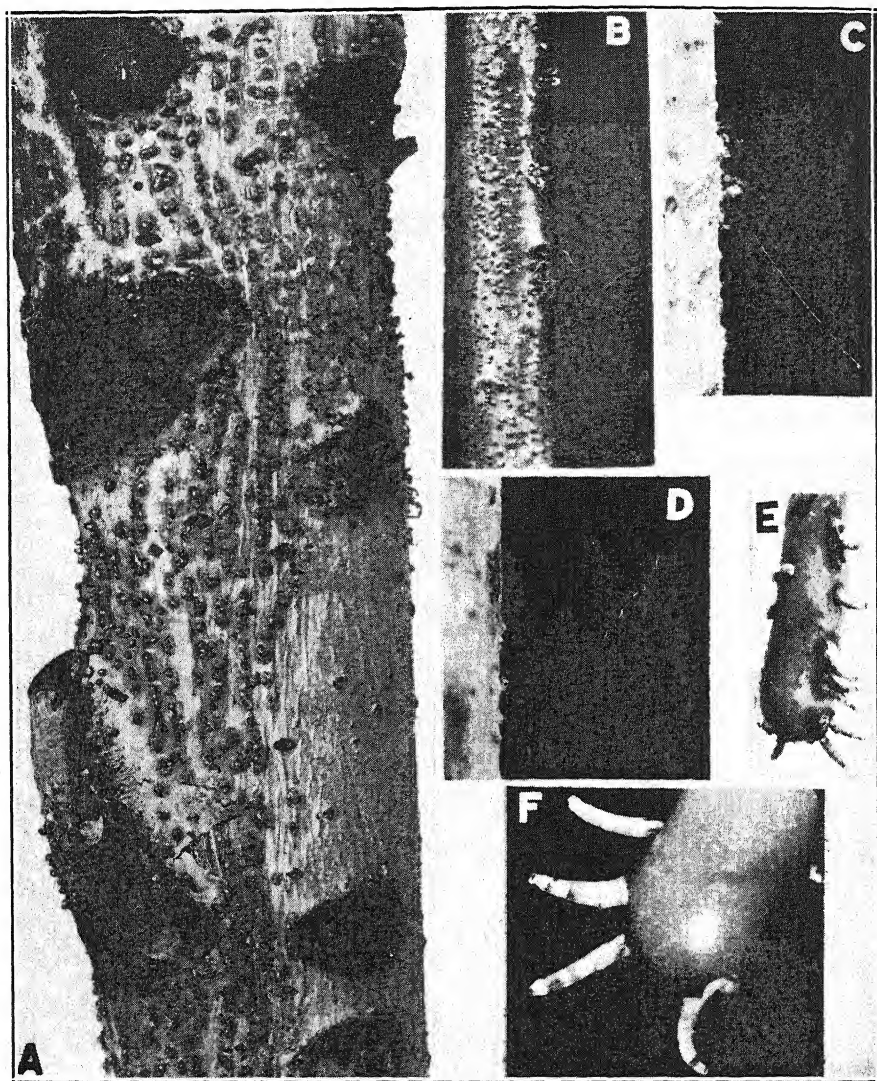


FIG. 3. A. *Cytospora chrysosperma* on poplar. B. On willow. Explained in text. C and D. Spore tendrils of *Cytospora chrysosperma* growing on aspen bark in a moist chamber. E and F. Pycnidia of *C. chrysosperma* on malt-agar slants in test tubes.

Growth on Agar Media. Cultures of most of the isolates produce pycnidia on malt agar, and the different isolates can be distinguished from each other readily by the size, shape, color, abundance, and time of production of pycnidia. While there is no one cultural character by which the species can be recognized, the production of pycnidia is typical of most isolates, although even individual variants of one isolate may differ greatly in this respect. Other species of *Valsa* and *Cytospora* also produce pycnidia of the same general character in culture, so that this alone can not be used as an identifying character.

Cultures of a few isolates, and particularly one of the variants of Isolate 1, produced peculiar, elongate pycnidia, several of which are illustrated in figure 3, E and F. Apparently, this development is due to the fact that the pycnidia arise in such a position that the tip presses against the wall of the test tube. The elongation of the pycnidium is equal to the shrinkage of the agar, and the continued pressure of the glass against the pycnidium stimulates growth in length. This was further illustrated by dropping a cover glass on top of 3 newly formed pycnidia on a culture in an Erlenmeyer flask. The 3 pycnidia under the cover glass continued to grow in length for several weeks after the other pycnidia in the culture had ceased to elongate visibly. This growth response to pressure obviously is a rather useful character from the standpoint of the fungus, since it is thus that the pycnidium is stimulated to break through the bark when growing on its natural hosts. Even when young pycnidia are covered by more than 20 layers of bark cells they are bound to break through, as they begin to elongate in the direction in which the bark gives way, continue to grow so long as pressure is exerted upon them, and cease growing soon after the pressure has been released.

All isolates were grown repeatedly on malt agar in Erlenmeyer flasks. The cultures of some isolates of *Cytospora chrysosperma* could not be distinguished from the cultures of some isolates of *Valsa sordida*. The variation between the different isolates of *C. chrysosperma* was greater than that between the isolates of *C. chrysosperma* as a group and those of *V. sordida* as a group, so that the two groups could not be separated by the cultural characters observed in this study. Similarity of cultural characters does not necessarily imply taxonomic identity, but it certainly does suggest that there is no very profound difference between *V. sordida* from *Populus tremuloides* and *C. chrysosperma* from other species of poplar.

Cultural Variation. Twenty single spores of Isolate 5 were isolated, and the cultures that developed from these were compared with each other and with the parent culture. When grown on agar in test tubes and flasks the different single-spore strains were remarkable chiefly for their uniformity of appearance; not more than two of them could be distinguished from the others or from the parent culture with any degree of positiveness.

It was observed that Isolate 8 in culture produced numerous pycnidia that differed from each other in size and shape. Mass spores were taken from several different pycnidia and grown separately, and the resulting cultures were found to be easily distinguishable from each other on the basis of the general appearance of the mycelium, time of formation of the pycnidia, and number and size of the pycnidia. Since it is not known how the pycnidia arise, and since the possibility exists that the original isolate may have been a mixture of strains, it is not known whether these obviously different strains were obtained merely by selecting previously existing strains from a mixed culture or whether they arose as variants when the parent culture was grown in the laboratory. No attempt was made to

pursue this phase of the problem further; it is mentioned only to illustrate the kind and extent of variation that may appear in cultures of this organism. Cultures of *Cytospora chrysosperma* from species of *Populus* and from some other host genera, and some of the variants obtained from these cultures are shown in figure 2, B.

PATHOGENICITY

Statements by Grove (1), Long (4), Hubert (2), Schreiner (9), and Povah (6) indicate that *Cytospora chrysosperma* can parasitize only more or less weakened trees. In Minnesota it undoubtedly is one of the most prevalent fungi fruiting on the bark of recently dead aspen in the forest, but only seldom has the writer found it apparently causing definite cankers on seemingly healthy, vigorous aspen growing in the forest. It seems doubtful if it is or will be of any considerable importance in naturally reproduced forests in this region. However, several nurserymen in Minnesota have expressed the opinion that the canker caused by this fungus is one of the greatest factors with which they have to contend in growing ornamental poplars. In 1936 one nursery reported 75 per cent of its stock of ornamental poplars killed by this fungus, and every one of the several nurseries visited by the writer in 1935 and 1936 suffered considerable loss of poplars, which the growers attributed to *Cytospora* canker. It is by far the most common fungus found on the dying and dead branches of ornamental poplars planted in this region, and frequently it forms definite cankers on the limbs of these trees. Such cankers have been assumed to be proof of the pathogenicity of the fungus, but they remain only circumstantial evidence.

Experimental Evidence of Pathogenicity

Hubert (2) inoculated 5 groups of poplar trees and cuttings with the fungus. One group contained 2 trees, another 3 trees, and the remaining groups 3 cuttings each. All inoculated plants except 2 trees died in from 3 to 6 months after having been inoculated, and pycnidia of *Cytospora* were formed on the dead bark. No checks were mentioned, and this, in addition to the small number of trees used, made the results of limited value. The fact that pycnidia of *Cytospora* were found on cuttings that previously had been inoculated with the fungus, does not prove that the fungus used was parasitic, or even that any parasitism was involved. The writer has found that pycnidia of *Cytospora* will develop, without inoculation, on cuttings of apparently healthy trees when the cuttings are placed in the laboratory or greenhouse and left for a few weeks. This will be referred to later.

Schreiner (9) inoculated 10 to 15 one- and two-year-old trees, growing in the field, each week from the first week in March until the end of May. In that same summer 50 per cent of the trees under $\frac{3}{4}$ in. diameter (presumably the weaker ones) and 5 per cent of the larger, more vigorous trees that he had inoculated were killed, supposedly by the fungus. Others died in succeeding years. Cuttings of several poplars were inoculated in the

greenhouse, and the dormant ones were found more severely injured than those that were growing.

On June 8 and 9, 1936, the writer inoculated 40 aspen saplings, each from 3 to 5 inches in diameter with 30 different isolates and single-spore cultures of *Cytospora chrysosperma*. Inoculations were made by macerating an area of bark about $\frac{1}{2}$ cm. square, placing a water suspension of spores and mycelium from an agar slant on this wound, covering this with a bit of moist cotton and wrapping it with paper tape. Only a very few small cankers were formed, and, even after 2 years, none of the inoculated trees had died. None of the check wounds became infected, perhaps because of the method of bandaging the wounds so that conditions favorable to the formation of callus tissue by the bark were maintained. On the other hand, typical *Cytospora* cankers developed around some of the wounds made in the bark when the trees were labelled by scratching numbers into the bark with a small chisel-like instrument, and these cankers doubtless originated from mycelium already present in the outer bark of the trees.

Natural Occurrence of *Cytospora* in the Bark of Healthy Poplars

During the course of the investigations a number of sections of several aspen trees were cut, brought into the laboratory, washed with hot tap water and soap, sponged with alcohol, then covered with a thin coat of hot paraffin. This treatment should have removed or killed any *Cytospora* spores on the surface of the bark. The logs were put in a fairly humid chamber, and, within 2 weeks, pycnidia of *C. chrysosperma* appeared over the greater part of the bark in such numbers that much of the paraffin layer was pushed off. Several cuttings from *Populus alba*, from trees that to all outward appearance were healthy, were placed in water in the laboratory, under much the same conditions, apparently, as the cuttings of *P. trichocarpa*, inoculated by Hubert. Two of these did not sprout, and both soon bore a very abundant crop of pycnidia of *C. chrysosperma*. The fungus doubtless was generally present in the bark of at least one of these, since pycnidia appeared everywhere on the surface at about the same time. A typical "canker" was formed on the other, if a canker can be said to form on a dead twig. Pycnidia of the fungus developed also on the dried tops of those cuttings that grew, and formed "cankers" there, but did not grow down from the dead tip, above the uppermost sprout, into the living tissue. Obviously, the fungus was not parasitic in this case. Eight branches of willow with no signs of *Cytospora* on the bark were brought into the laboratory in January, washed with soap and warm water, sponged with 70 per cent alcohol, covered with warm paraffin, and placed under a bell jar with the lower ends in a glass of water. *Cytospora* fruited abundantly on all of them. No inoculation was necessary. The fungus already was present, and became evident only after the twigs died. A fairly typical one of these was photographed (Fig. 3, B). A section of a living branch from a mountain ash (*Sorbus aucuparia*) was brought into the laboratory at the same time and placed

under a bell jar, with the lower end of the section in water. After almost a month had elapsed, no pycnidia of *Cytospora* had appeared on this branch; the bark, when cut into, appeared green and still living. The branch then was placed outside for several hours, until the bark was frozen sufficiently to kill it, and again placed under the bell jar. Within a week typical sunken, discolored cankers appeared on the bark of this branch, and pycnidia of *Cytospora* appeared throughout these "cankers." In this case the fungus, although present, was unable to produce any visible effects even in bark that must have been on the verge of death, and only after the bark was quite dead could the fungus produce cankers and fruit bodies.

A group of *Populus alba* trees was planted on the campus in the spring of 1938, located where the writer passed them almost every day and thus had a good opportunity to observe them closely. Three of these trees died within about 4 months after being planted. Although there was no sign of *Cytospora* canker on the trees when the first inconspicuous symptoms of impending death became evident, pycnidia appeared generally throughout the bark of trunk and branches of one of them soon after the leaves became dry. This tree was cut and the base of the trunk placed in water, with the result shown in figure 3, A. One who examined the tree for the first time after *Cytospora* had appeared could have supposed the fungus to have killed it. *Cytospora* pycnidia appeared on some of the limbs of the other two dead trees. Several limbs of these two trees, none of them with any evidence of pycnidia, were placed with one end in water and within two weeks a most abundant crop of pycnidia was produced on the bark. When in need of good specimens of *Cytospora* to show to his students, the writer merely cuts branches of ornamental poplars or of forest willows, places them in water, and always obtains excellent specimens of the fungus. No inoculation is necessary. The fungus appears to be generally present in the healthy bark of poplars, willows, and some other species of trees in this region.

The foregoing observations and experiments have made the writer somewhat dubious of the actual parasitism involved in many cases of canker formation by *C. chrysosperma*. Sunken areas formed in the bark where the fungus is growing vigorously may be due chiefly to the fact that the fungus has digested portions of the tissues—it does not mean that the nonsunken portion surrounding the canker necessarily is healthy. Thus, when twigs are placed in water, the fungus forms a canker if it is only of local extent in the bark, otherwise it may fruit all over the surface of the twig without forming any definite canker.

There is no doubt that most of the clones of ornamental poplars introduced from Europe and grown in Minnesota are poorly adapted to the soil and climate here. Observational and even experimental evidence indicates that *Cytospora chrysosperma* attacks weakened trees, but if it is generally present in the bark of trees so ill adapted to their environment as many aspen and native willows on poor sites, and many introduced varieties and

clones of ornamental poplars and mountain ash in this region, it must be a weak parasite indeed to permit such trees to survive at all. The writer certainly considers it an open question whether many of the trees seemingly parasitized by *C. chrysosperma* would survive were the fungus not present. The fact that many ornamental poplars succumb the first or second year after they have been transplanted would also tend to emphasize the question as to whether, at least in this region, they die because they are invaded by *C. chrysosperma* or whether *C. chrysosperma*, normally present in the bark, fruits on them because they are dying. The writer does not wish to imply that he believes *C. chrysosperma* unable ever to grow as a parasite, but certainly at times it has been incriminated on insufficient evidence. The writer is of the opinion that the poor survival of ornamental poplars in this region is due less to *Cytospora* than to the fact that these poplars are exotic trees growing in soils and subjected to climatic conditions to which they simply are not adapted. The practical conclusion to be drawn from this is that, so far as the control of *Cytospora* canker on ornamental poplars is concerned, it would be far more to the point to try to develop varieties better adapted to the locality in which they are to be grown, rather than to seek specific prophylactics or remedies for this disease alone.

DISCUSSION

It was not possible to find any consistent difference in shape, size, or structure of the pycnidia, or shape, size, or manner of production of conidia between *Valsa sordida*, from aspen, and *Cytospora chrysosperma* from other species of poplar. At no time could the cultures of the different isolates of *C. chrysosperma* as a group be distinguished from the cultures of isolates of *V. sordida* as a group. There were constant and characteristic differences between different isolates, as perhaps would be expected, but the cultures of some isolates of *C. chrysosperma* were almost identical with those of some isolates of *V. sordida*. From these results it seems fairly safe to state that those strains of *C. chrysosperma* growing on species of *Populus* other than *P. tremuloides* fall within the range of variability, in the characters studied, of the imperfect stage of *V. sordida*. The writer has examined, but has not studied thoroughly, the morphologic and cultural characters of some isolates of *Cytospora* from elm, walnut, willow, and a few other hosts and, at present, he has found no good basis for separating these from *C. chrysosperma* on *Populus*.

Cytospora chrysosperma varies considerably in most of the characters studied. The length of conidia seems to vary within relatively narrow limits, but the conidia of what are considered to be other species of *Cytospora* fall within these limits, so that this character alone is not a distinguishing character—or some of the species are not valid. The shape and microscopic structure of the pycnidia, though rather variable, are fairly characteristic, but *Cytosporas* considered to be other species have essentially the same structure. The manner in which the pycnidial stroma of *C.*

chrysosperma is borne in the bark of the host is typical of a large number of what have been considered other species. The spore tendrils are supposed to be rather large and yellow, but this depends greatly on environmental conditions. Fresh tendrils usually are yellow, but the writer has found *C. chrysosperma* on *Populus alba* and *P. nigra* with red spore tendrils, and when these were grown in culture the tendrils were yellow. Spores of both *C. chrysosperma* and *V. nivea* are exuded from old pycnidia in white masses, so evidently the color fades. The essence of the foregoing is that the writer has not yet been able to find any good diagnostic character of the species.

The fact that the fungus is a more or less normal, though unseen, inhabitant of the bark of apparently healthy poplar, willow, and mountain ash trees, and does not fruit until the trees are dying, makes it difficult to judge how much of a factor it may be in the death of these trees. The writer believes he has a fairly good basis for suggesting that the fungus often is not responsible for the injury with which it is associated.

SUMMARY

The pycnidia and conidia of *Valsa sordida* developed in the field and in the laboratory could not be distinguished from those of *Cytospora chrysosperma* produced under comparable conditions.

Isolates of *V. sordida* and *C. chrysosperma* differed among themselves in culture, but there was no consistent difference between those of the former as a group and those of the latter as a group.

Collections of *Cytospora* from hosts other than *Populus* could not be distinguished from collections of *C. chrysosperma* from species of *Populus* when compared as to structure of pycnidia, rate of growth on agar, and general cultural characters.

C. chrysosperma is a common inhabitant of the bark of apparently healthy *Populus* trees, especially *P. tremuloides* and *P. alba*, and probably also of willow and mountain ash. The degree of its parasitism on these trees is considered open to question.

It is suggested that the problem of control of *Cytospora* canker on ornamental poplars should be approached by attempting to develop varieties of trees more suited to their general environment than the present ones.

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THE CHEMISTRY OF RESISTANCE OF PLANTS TO PHYMATOTRICHUM ROOT ROT. V. INFLUENCE OF ALKALOIDS¹ ON GROWTH OF FUNGI²

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INTRODUCTION

The presence of alkaloids in plants has been suggested (5) as an important factor in the mechanism of their resistance to *Phymatotrichum omnivorum* (Shear) Duggar. This was done by correlating the presence or absence of alkaloids in plants with resistance of the species to the fungus as reported by Taubenhaus and Ezekiel (16). This view was strengthened by demonstrating that *Mahonia trifoliolata* Fedde, *M. swaseyi* Fedde and *Sanguinaria canadensis* L. contained large quantities of alkaloids toxic to the fungus and so located in the plant as to account fully for the resistance of the plant (6, 7). To further test the hypothesis, 62 different alkaloids from plants differing in their susceptibility to *P. omnivorum* have been studied by incorporating them, in various concentrations, into the substrates of the medium ordinarily used for pure culture of the organism. In addition, for comparative purposes, the influence of 6 alkaloids on 6 fungi other than *P. omnivorum* has been determined.

LITERATURE REVIEW

The influence of alkaloids on the growth of fungi has been the subject of several investigations. Bates (1) studied the effect of strychnine, quinidine, and caffeine on the growth of *Aspergillus niger* and *Rhizopus nigricans*. He used concentrations of the alkaloids up to 1.8 per cent. Bates found that strychnine sulphate increased growth of *R. nigricans* and of *A. niger* in Coons nutrient solution, and quinidine sulphate had a similar effect on the latter organism. Growth of both fungi was decreased by caffeine citrate and that of *R. nigricans* by quinidine sulphate. These 3 compounds also retarded sporulation of *A. niger*.

Marcacci (9) found that lactic acid fermentation was accelerated by the presence of atropine and morphine but was retarded by quinine, veratrine,

¹ For the sake of convenience, the term "alkaloid" is used in its broader sense to include the purines and some of the simpler naturally occurring nitrogen bases.

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cinchonamine, and strychnine, the last being the most active. All of the above alkaloids produced a favorable action on alcoholic fermentation except cinchonamine and quinine.

Botrytis cinerea Pers. was cultivated by Nobecourt (10) on Raulins liquid containing varying amounts of nicotine and sulphates of atropine, quinine, and aconitine. He found that nicotine sulphate in a concentration of .025 M and atropine sulphate in a concentration of .020 M did not hinder the growth of this fungus. Growth was not retarded by .010 M of quinine sulphate, but a concentration of .020 M resulted in small thalli with few conidiophores, while one of .030 M prevented growth. Growth was visibly hindered by aconitine in a concentration of .002 M and greatly reduced by a concentration of .004 M. A concentration of .010 M did not prevent the germination of spores, which was completely inhibited at .020 M.

Smith (12) reported that *Botrytis cinerea* grew very poorly in 1 per cent solutions of brucine and strychnine and that no growth occurred in a 2 per cent solution of quinine and caffeine. Ravaz and Gouirand (11), however, found that this fungus grew normally at 0.1 per cent concentration. The effect of caffeine, quinine, and nicotine on the germination of *Botrytis cinerea* spores was studied by Staritzky (14). He found no germination at 0.1 per cent and normal germination at .001 per cent of caffeine; normal germination with .001 per cent quinine sulphate; no germination at .001 per cent, and normal germination at .0001 per cent of nicotine.

Yasuda (17) found that the growth of *Penicillium glaucum*, *Aspergillus niger*, *Botrytis cinerea*, and *Mucor stolonifer* was increased by the addition of the hydrochlorides of cocaine, quinine, cinchonine, morphine, codine, and strychnine to Richards nutrient solution. The alkaloids were used in concentrations varying from 0.2 to 2 per cent. As the concentration of alkaloid was increased, the conidiophores and sporangiophores became thinner and shorter. Formation of conidia and sporangia was entirely suppressed and replaced by that of chlamydospores when the optimum concentration for fungus-vegetation was surpassed. The weakest alkaloid for the fungi under consideration was the hydrochloride of morphine, while the strongest was that of cocaine. The fungi listed in order of their decreasing resistance to alkaloids are: *P. glaucum*, *A. niger*, *B. cinerea* and *M. stolonifer*.

Ehrlich (2) grew *Oidium lactis*, *Aspergillus niger*, *Penicillium glaucum*, *Willia anomala*, *Pichia farinosa*, a mixed culture obtained by exposing the culture solution to the air, and an unknown species of wine yeast on a mineral nutrient solution to which he added different alkaloids as the only source of nitrogen and ethyl alcohol or invert sugar as the source of carbon in concentrations of 0.2 and 2 per cent, respectively. A control series without the alkaloid was run at the same time. The alkaloids used at 0.2 per cent were: pyridine, piperidine, coniine, nicotine, cinchonine, quinine, brucine, cocaine and morphine. Ehrlich obtained only a small amount of growth, least in the yeast cultures and greatest in the mixed cultures. The retardation of growth he obtained was attributed to the poisonous action of the decomposition products of the alkaloids.

Enders and Wieninger (3) noted the effect of alkaloids on fermentation and multiplication of yeast. They found that the toxicity of alkaloids for yeast (as measured by the inhibition of fermentation and multiplication) diminished in the order: quinine, caffeine, cinchonine and pilocarpine. In general, much higher concentrations of alkaloids were required to inhibit the power to multiply than to inhibit fermentative ability.

MATERIALS AND METHODS

Certain of the alkaloids used in this investigation were secured from the following: R. H. Manske, National Research Council, Ottawa, Canada; James F. Couch, Bureau of Animal Industry, Washington, D. C.; W. M. Neal, Florida Agricultural Experiment Station, Gainesville, Florida; H. Kondo, Imperial University, Tokyo, Japan; J. Madinaveitia, University of Edinburgh, Edinburgh, Scotland; Karl Folkers, Merck and Co. Inc., Rahway, New Jersey. The courtesy of these investigators in providing alkaloids of their own isolation and purification is greatly appreciated. The alkaloids from *Mahonia*, *Berberis*, and *Sanguinaria* species were isolated and purified in this laboratory. The remainder were obtained from commercial sources and used without further purification.

The effect of the alkaloids on the growth of *Phymatotrichum omnivorum*, *Sclerotium rolfsii* Sacc., *Fusarium vasinfectum* Atk., *Verticillium albo-atrum* McA., *Rhizoctonia solani* Kühn, *Armillaria mellea* (Vahl.) Quel. and *Ophiobolus graminis* Sacc. was tested by growing the fungi on a liquid medium to which the alkaloid had been added in concentrations from .01 M to .0001 M. The nutrient solution³ consisted of MgSO₄, 0.75 g.; K₂HPO₄, 1.35 g.; NH₄NO₃, 1.00 g.; KCl, 0.15 g.; and dextrose, 40.00 g. per l. After removal of heavy metals by the method of Steinberg (13), Mn⁺⁺, Fe⁺⁺, Cu⁺⁺, and Zn⁺⁺ were added at concentrations of 2.5 p.p.m. This solution is a modification of the nutrient solution 70 of Ezekiel, Taubenhaus, and Fudge (4) and has been shown by Talley and Blank (15) to give a proper supply and balance of the major and minor elements necessary for this organism. The pH of the final nutrient solution was approximately 6.5.

The necessary amount of each alkaloid was added to sterile 250-ml. Florence flasks. Usually this was done by pipetting the proper quantity of a 95 per cent ethyl-alcohol solution of the compound into the flask; sometimes, however, it was more convenient to weigh the alkaloid directly into the flask and add one ml. of alcohol for sterilization afterward. After the alcohol had evaporated, to each flask was added 25 ml. of sterile nutrient solution. Thus the opportunity for error due to heating chemicals and nutrient solution in presence of each other was avoided. The inoculum for each flask consisted of a disc 6 mm. in diameter cut from a nutrient agar plate covered with the mycelium of the fungus. The average dry weight of each disc was 2.5 to 3 mg. The cultures were incubated at 28° C. for the

³ In one experiment with *Sclerotium rolfsii* and *Ophiobolus graminis*, potato dextrose medium was used in addition to the standard solutions reported.

following lengths of time shown by preliminary experiments to yield approximately maximum mat weights: *Sclerotium rolfsii*, 4 days; *Fusarium vasinfectum* and *Rhizoctonia solani*, 7 days; *Verticillium albo-atrum*, 10 days; *Phymatotrichum omnivorum* and *Ophiobolus graminis*, 21 days; *Armillaria mellea*, 28 days. After incubation, the fungus mats were removed by means of a hooked rod, washed with distilled water, dried to constant weight at 80° C. and weighed on an analytical balance. Each value reported is the average obtained from six cultures inoculated at three successive times, or, in a few instances, in triplicate at two different periods. The control values were obtained from six flasks without alkaloids run at each replication.

EXPERIMENTAL RESULTS

The results of growth studies on *Phymatotrichum omnivorum*, *Sclerotium rolfsii*, *Fusarium vasinfectum*, *Verticillium albo-atrum*, *Rhizoctonia solani*, *Armillaria mellea* and *Ophiobolus graminis* in nutrient solution to which alkaloids were added in concentrations of .01 M to .0001 M are presented in table 2. In table 1 are listed 62 alkaloids in order of their decreasing toxicity to *P. omnivorum* as measured by inhibition of mat weight. Although molar concentrations have been used, the p.p.m. for .0001 M concentrations are listed for convenience in making comparisons on this basis.

A study of the data in table 1 reveals the fact that certain alkaloids are highly toxic to *Phymatotrichum omnivorum* at low concentrations, while others are not inhibitory at high concentrations. At the lower concentrations of some of these compounds there are indications of a slight stimulation of growth over that of the controls. The results indicate that this fungus may use the nitrogen base xanthine as a source of nitrogen, since the yields were 468 mg., 437 mg., and 419 mg., respectively, as compared with 397 mg. for the controls, for the concentrations of .01 M, .001 M and .0001 M.

Toxicity of Alkaloids to Other Fungi

As indicated earlier in the paper, the study of certain of the alkaloids was extended to include *Sclerotium rolfsii*, *Ophiobolus graminis*, *Rhizoctonia solani*, *Armillaria mellea*, *Fusarium vasinfectum*, and *Verticillium albo-atrum*. These data are presented in table 2. The order of decreasing ability of these fungi to tolerate the alkaloids are: *V. albo-atrum*, 1.7⁴; *F. vasinfectum*, 2.0; *R. solani*, 3.5; *A. mellea*, 4.2; *O. graminis*, 4.8; *S. rolfsii*, 5.5; *P. omnivorum*, 6.3. The order of toxicity of the compounds to the 7 fungi are: sanguinarine 1.1, delphinine 2.4, berberine 4.1, gramine 4.3, solanine 4.3, veratrine 4.7.

DISCUSSION

Although a compound must contain nitrogen in a ring to be classified as an alkaloid, the other groups present exert a profound influence upon

⁴ The values given are the average relative order of each of the 7 fungi in ability to tolerate the alkaloids. The values for alkaloids are the average order of each in toxicity to the fungi.

the physiological behavior of the molecule. Unfortunately, the compounds tested are so complex and the proportion of the total number of possible compounds so small that it is not possible at this time to make any broad generalizations regarding the relationship between toxicity and chemical or physical properties. Nevertheless it is of interest to note the results obtained with several groups of related compounds. For example, the addition of methyl groups to xanthine, to form theobromine (3, 7-dimethylxanthine) and caffeine (1, 3, 7-trimethylxanthine) increases progressively the toxicity to *Phymatotrichum omnivorum*. Hypaphorine, the methylbetaine of tryptophan, was found to inhibit greatly the growth of *P. omnivorum* at a concentration of .005 M. Brucine, dimethoxystrychnine, is more toxic than strychnine itself. The influence of methyl and methoxy groups attached to the benzene ring upon the growth of *P. omnivorum* is reported elsewhere (8).

All plants contain basic nitrogenous compounds, protein degradation products, choline, betaine, or similar substances that will react with certain of the alkaloidal reagents. However, the occurrence of alkaloids in the accepted use of this term is confined to a rather restricted number of botanical groups. Some of the large groups of plants do not so metabolize their nitrogen as to yield alkaloids. This is true of many of the genera of the Labiatae and Compositae, although there is one outstanding exception in the genus *Senecio* of the Compositae. The grasses are not characteristically alkaloid-bearing. Although recent studies have revealed that gramine is present in *Arundo donax* and *Hordeum vulgare* var. Chevalier I and II, Primus I and II, Gold \times Chevalier, etc.⁵; loline in *Lolium temulentum*; an unidentified alkaloid in *Oryza sativa*. Alkaloids isolated from other monocotyledonous plants are lycorine from some twenty species of the Amaryllidaceae; veratrine from *Veratrum sabadilla*, *V. album*, *V. lobelianum*, *V. viride*, and *V. nigrum*. A number of the Liliaceae genera have yielded several alkaloids, e.g., *Fritillaria* and *Zygadenus*. The palms, with the exception of the areca or betal palm, are nonalkaloidal plants. In general, alkaloids are yielded by such important families as the Ranunculaceae, Berberidaceae, Papaveraceae, Fumariaceae, Leguminosae, and Solanaceae; these families contain species resistant to *Phymatotrichum* root rot.

These data (Table 1) furnish information on 50 to 70 species of plants from 15 families that differ in their susceptibility to *Phymatotrichum omnivorum*. The relative toxicity of these alkaloids to *P. omnivorum* follows in general the relative resistant rating of the plant from which they have been isolated. The correlation between the presence of alkaloids in plants and their resistance rating to *P. omnivorum* has been published (5).

The toxicity of alkaloids to this fungus does not correspond to their relative toxicities for the animal organism. A similar observation on the influ-

⁵ Brandt, K., H. V. Euler, et al. Hoppe-Seyl. Zeitschr. Phys. Chem. 235: 37-42. 1935. They found that the presence of gramine was correlated with the resistance of barley varieties to nematodes. Hordenine also has been isolated from barley, and it is identical, according to Späth, with anhaline from *Anhalonium fissuratum* (Cactaceae).

TABLE 1.—*Influence of alkaloids on the growth of Phymatotrichum omnivorum*

Compound ^h	Dry wt. (mg.) fungus grown in nutrient solution plus the following concentrations of alkaloids					P.p.m. of free base at .0001 M conc.
	.01 M	.005 M	.001 M	.0005 M	.0001 M	
Sanguinarine ^a	0	0	0	0	0 ⁱ	35.1
Sanguinarine ^a SO ₄	0	0	0	0	0	35.1
Chelerythrine HCl ^a	0	0	0	0	0 ⁱ	41.1
Lycorine ^b	0	0	0	0	28 ^j (14-74)	28.7
Oxycanthine ^a	0	0	0	139 (120-159)	208 (185-256)	31.1
Delphinine	0	0	0	155 (139-174)	310 (221-402)	57.7
Berbamine ^a	0	0	0	162 (145-201)	268 (214-341)	33.3
Berberine HCl ^a	0	0	0	175 (159-192)	316 (307-319)	44.3
Quinine	0	0	29 (15-46)	211 (193-231)	339 (329-363)	32.4
Veratrine	0	0	65 (18-74)	223 (194-250)	356 (301-419)	59.1
Gramine (donaxine) ^c	0	28 (17-41)	158 (129-171)	241 (190-253)	213 (198-229)	17.2
Protopine ^d	0	75 (63-91)	138 (101-169)	300 (280-311)	350 (340-365)	35.3
Lobeline SO ₄	0	85 (67-103)	161 (136-190)	305 (293-319)	346 (311-397)	32.1
Spartiodine ^d ...	0	99 (86-112)	172 (133-278)	334 (309-380)	33.3
Integerri- mine ^d	0	102 (85-121)	186 (165-225)	339 (317-346)	33.5
Quinidine	0	109 (89-117)	295 (276-315)	369 (360-389)	36.9
Aspidosper- mine	0	115 (99-132)	233 (211-240)	364 (349-384)	35.4
Ephedrine	0	118 (97-137)	214 (183-255)	381 (374-386)	18.3
Cinchonine HCl	0	124 (110-134)	285 (169-330)	385 (368-421)	29.4
Nicotine	0	142 (107-159)	307 (240-394)	340 (315-360)	16.2
Scoulerine ^d	0	145 (123-169)	308 (291-326)	344 (336-351)	32.7
Caffeine	0	149 (139-163)	327 (240-388)	363 (300-401)	19.4
Hypaphorine ^e	0	151 (120-169)	314 (283-328)	360 (327-386)	28.2
Retrorsine ^d	0	154 (129-180)	310 (225-357)	347 (329-374)	35.1
Monocrota- line ^f	0	159 (139-182)	311 (287-377)	360 (336-407)	32.8

TABLE 1.—(Continued)

Compound ^h	Dry wt. (mg.) fungus grown in nutrient solution plus the following concentrations of alkaloids					
	.01 M	.005 M	.001 M	.0005 M	.0001 M	P.p.m. of free base at .0001 M conc.
Lupinines ^e	0	160 (139-190)	311 (271-387)	374 (340-444)	35.2
Eserine (physostigmine)	0	163 (143-182)	315 (269-382)	370 (349-401)	27.5
Atropine	0	164 (134-192)	320 (301-352)	369 (326-435)	29.8
Sparteine SO ₄ ^g	0	165 (136-194)	339 (309-387)	357 (339-386)	23.4
Pelletierine SO ₄	0	166 (146-187)	316 (277-366)	379 (350-406)	14.1
Corlumined ^d	0	198 (173-210)	322 (296-340)	382 (344-415)	38.3
Corydine HCl ^d	0	200 (183-215)	323 (296-344)	363 (329-398)	36.9
Deltalines ^e	0	298 (283-315)	365 (320-445)	384 (344-453)	39.6
Betaine HCl ...	0	303 (289-314)	374 (315-484)	419 (397-436)	11.7
Tryptamine	0	320 (300-359)	374 (341-400)	408 (389-421)	16.0
Brucine	4 (0-7)	333 (319-356)	349 (311-397)	389 (349-422)	46.6
Bieucullined ^d ...	7 (0-13)	333 (310-357)	372 (322-414)	361 (337-370)	36.7
dl-lupanines ^e	20 (6-70)	339 (310-360)	346 (318-400)	404 (376-450)	24.8
l-tetrahydro-palmatined ^d	42 (7-68)	340 (320-371)	314 (274-365)	353 (346-368)	35.5
Bieucined ^d	56 (37-67)	341 (316-351)	379 (297-416)	371 (329-404)	38.5
Ochotensined ^d ...	126 (102-137)	338 (327-342)	335 (323-340)	380 (369-391)	35.1
Strychnine HCl	137 (105-194)	339 (291-381)	363 (337-402)	33.4
Cocaine HCl ...	142 (132-150)	321 (311-327)	368 (349-418)	30.3
Procaine HCl	185 (173-211)	352 (338-399)	367 (346-394)	23.6
α-erythro-idine ^e	211 (183-248)	334 (287-365)	347 (294-364)	27.3
Trilupines ^e	242 (196-336)	357 (333-411)	401 (376-456)	31.6
Histamine di HCl	271 (258-294)	385 (343-419)	422 (411-439)	11.1
Tyramine HCl	273 (263-288)	364 (353-378)	429 (382-470)	13.7
Hyoscine HCl	288 (260-305)	351 (344-388)	361 (348-385)	30.3

TABLE 1.—(Continued)

Compound ^b	Dry wt. (mg.) fungus grown in nutrient solution plus the following concentrations of alkaloids					P.p.m. of free base at .0001 M conc.
	.01 M	.005 M	.001 M	.0005 M	.0001 M	
Theobromine	306 (296–318)	356 (349–360)	407 (375–455)	18.0
Choline HCl ...	322 (311–330)	387 (286–425)	389 (283–423)	12.1
Colchicine	331 (302–377)	373 (326–455)	406 (365–451)	39.9
Capaurine ^d	336 (304–374)	355 (341–370)	370 (360–385)	37.3
Capauridine ^d	337 (332–341)	354 (346–360)	353 (341–365)	37.3
Hyosecyamine HCl	339 (326–349)	372 (355–399)	358 (331–401)	28.9
Hydrastine	343 (323–356)	360 (329–397)	373 (336–419)	38.3
Homatropine	358 (339–382)	375 (337–407)	384 (341–411)	27.5
Aconitine	359 (310–392)	351 (322–374)	350 (337–360)	64.7
Adrenaline { animal	393	429	439	18.3
{ alkaloid	(369–416)	(401–457)	(430–448)	
Xanthine	468 (450–473)	437 (420–446)	419 (410–435)	15.2
Controls = Av. 397 (346–452)						

^a Isolated and purified in U.S.D.A. Plant Physiology Laboratory, College Station, Texas.

^b Donated by Dr. H. Kondo.

^c Purchased from Dr. J. Madinaveitia.

^d Donated by Dr. R. H. Manske.

^e Donated by Dr. Karl Folkers.

^f Donated by Dr. W. N. Neal.

^g Donated by Dr. James Couch.

^h Solanine (glucoside-alkaloid) and zygademon alkaloids were tested at 0.1%; .01%; and .001% and yielded 0 mg., 14 mg. and 354 mg.; 0 mg., 19 mg. and 252 mg., respectively.

ⁱ Sanguinarine was found to completely inhibit the growth of the fungus at 2.5 p.p.m.; chelerythrine tested under similar conditions yielded 3.4 mg. fungus mat at 10 p.p.m. (6).

^j The first number given is the average value of 6 cultures, while the figures in parenthesis show the range of growth, i.e., the minimum and maximum values obtained.

ence of quinine, caffeine, cinchonine, and pilocarpine on the fermentation and multiplication of yeast has been recorded by Enders and Wieninger (3).

The data (Tables 1 and 2) of this investigation indicate clearly that it is prudent to reserve judgment on the protective rôle of an alkaloid until it has been isolated from the host tissue in the pure form. The concentration and localization, as well as the toxicity of the compound to the parasite in ques-

TABLE 2.—Influence of certain alkaloids on the growth of seven fungi. The results are calculated as the percentage of the controls

Cul- ture me- diu m	Incu- ba- tion period days	Dry weight (mg.) of fungi grown at various molar concentrations of compound, calculated as percentage of control											
		Sanguinarine SO ₄			Berberine HCl			Veratrine			Gramine		
		Con- trols	.01	.001	.0001	.01	.001	.0001	.01	.001	.0001	.01	.001
No. P.d. 70	4 21	160 ^a 115	0 0	0 0	0 0	0 0	0 0	3.8 0	11.3 0	22.5 39.1	55.6 90.4	0 0	41.3 53.0
70	21	397	0	0	0	0	0	79.6	0	16.4	89.7	0	53.7
P.d.	21	108	0	0	0	3.7	56.5	90.7	0	76.9	86.1	0	81.5
70	7	496	0	0	4.8	13.5	75.8	99.0	24.3	94.2	99.2	0	93.8
70	28	102	0	11.8	33.3	0	47.1	65.7	0	18.6	92.2	0	40.2
70	7	232	0	0	18.5	59.9	88.4	96.1	29.7	103.9	102.2	11.2	96.1
70	10	367	0	0	9.0	73.3	96.2	101.6	0	104.9	99.7	15.8	103.3

a Abbreviations used in this table are: S.r. = *Sclerotium rolfsii*; P.o. = *Phymatotrichum omnivorum*; O.g. = *Ophiobolus graminis*; R.s. = *Rhizoctonia solani*; A. = *Armillaria mellea*; F.v. = *Fusarium vasinfectum*; V.a. = *Verticillium albo-atrum*. R.s., S.r., and A.m. were secured from W. N. Ezekiel; F.v. and V.a. from O. Sherbakoff; O.g. from Hurley Fellows; P.o. isolate No. 28 from L. M. Blank.

b P.d. = Potato dextrose; No. 70 = standard solution described in text.

c Since replicates within a treatment were average in uniformity, the range of mat weights was omitted.

tion, should be determined before predicting the possible relation of a given compound to the resistance or immunity of a plant.

SUMMARY

Sixty-two different alkaloids from 15 families and 50 to 70 species of plants differing in their susceptibility to *Phymatotrichum* root rot have been studied as to their influence on the growth of this fungus. Sanguinarine was found to be the most toxic alkaloid studied. It completely inhibited the growth of *Phymatotrichum omnivorum* at a concentration of 2.5 p.p.m. The alkaloids are listed in order of their decreasing toxicity, on a molar basis, to *P. omnivorum*: Sanguinarine, chelerythrine, lycorine, oxyacanthine, delphinine, berbamine, berberine, quinine, veratrine, gramine, protopine, lobeline, spartiodine, integerrimine, quinidine, aspidospermine, ephedrine, cinchonine, nicotine, scoulerine, caffeine, hypaphorine, retrorsine, monocrotaline, lupinine, eserine, atropine, sparteine, pelletierine, corlumine, corydine, delta-line, betaine, tryptamine, brucine, bicuculline, dl-lupanine, l-tetrahydropalmatine, bicucine, ochotensine, strychnine, cocaine, procaine, α -erythro-*id*ine, trilupine, histamine, tyramine, hyoscyne, theobromine, choline, colchicine, capaurine, capauridine, hyoscyamine, hydrastine, homatropine, aconitine, adrenaline, xanthine.

The influence of 6 alkaloids on growth of *Phymatotrichum omnivorum*, *Sclerotium rolsii*, *Ophiobolus graminis*, *Armillaria mellea*, *Rhizoctonia solani*, *Fusarium vasinfectum*, and *Verticillium albo-atrum* in liquid culture were studied, and it was found that the fungi show increasing ability to tolerate alkaloids generally in the order given. Although the fungi reacted differently to different alkaloids, the order of decreasing potency among the compounds tested was sanguinarine, delphinine, berberine, gramine and solanine, and veratrine.

In general, the relative toxicity of the alkaloids studied to *Phymatotrichum omnivorum* follow the relative resistant rating of the plant from which they were isolated. This indicates that certain alkaloids in roots of plants constitute an important factor in the resistance of these plants to *P. omnivorum*.

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TOXICITY OF PARADICHLOROBENZENE IN RELATION TO CONTROL OF TOBACCO DOWNY MILDEW¹

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INTRODUCTION

Experimentation involving the use of organic compounds to control tobacco downy mildew has now been in progress for several years. Empirical methods were used in our early experiments (3) with fumigants; but, gradually, a better understanding of the principles underlying their use has been evolved (5, 8). These studies have shown that volatile materials may be more effective fungicides than the nonvolatile ones ordinarily employed as dusts or sprays. It seems probable that the greater efficacy of volatile materials is related to their action not only as protectants but also as eradicants. This latter mode of action is novel in the field of plant pathology and is dependent upon the ability of volatile substances to penetrate infected leaves and either to inhibit the development of the pathogen or to be lethal to it, *in situ*, without apparent injury to the host tissues.

Although our studies have been concerned primarily with benzol, other volatile materials have been tested and found to have a similar mode of action. Prominent among those that have given promise of success is paradichlorobenzene, $p\text{-C}_6\text{H}_4\text{Cl}_2$. This volatile crystalline product has long

¹ Cooperative investigations conducted by the Virginia Agricultural Experiment Station and Duke University.

been used as an insecticide, especially against peach-tree borers and clothes moths. Apparently, no attempts were made to employ paradichlorobenzene (hereafter called PDB) to control plant diseases prior to 1936, when it was used in seedbeds as a fumigant against downy mildew (3). In these experiments the crystals of PDB were placed in pans resting on the soil amongst infected plants, but without appreciable beneficial effect. Two years afterward the late W. M. Lunn, of Florence, South Carolina, observed that, in seedbed experiments, PDB had considerable promise as a control agent of this tobacco disease, and encouraged investigations by others with this material. Subsequently, reports by Clayton (1) and Pinckard and McLean (6) appeared. The most important results from the work of Pinckard and McLean (6) were that they called attention to failure to control downy mildew if ordinary seedbed covers are used for retaining PDB vapor within the bed, that best control occurs if the area of the surface on which the crystals are distributed to be vaporized is equal to that of the seedbeds, and that this compound becomes an eradicant fungicide if used in sufficient concentrations.

It appeared desirable to learn what concentration of PDB could be tolerated by tobacco seedlings on the one hand, and what strength was toxic to the tobacco downy-mildew fungus on the other. No methods were available by which this could be accomplished, since the usual methods of evaluating the fungicidal or germicidal properties of chemical substances are manifestly of little value when applied to gaseous fungicides. It became necessary, therefore, to devise, first of all, a laboratory method for testing the fungicidal value of PDB, to demonstrate the accuracy of this method, and finally to initiate a series of laboratory studies to determine the minimal concentration of this fumigant that can be safely used against tobacco downy mildew.

The present report embodies the results of these studies with PDB that are deemed basic to experimentation involving tobacco plants growing in seedbeds. In addition it discusses a general procedure applicable to investigation of volatile fungicides.

APPARATUS AND METHODS

Laboratory studies on the fungicidal value of volatile substances would appear to be most useful and the results would seem best to serve as the basis for subsequent field experiments if both the host and parasite could be acted upon simultaneously by the chemical under consideration. It would appear, furthermore, that an apparatus constructed to accomplish this purpose should possess the following features: It should be so constructed as (a) to insure a controlled concentration, in the air, of the vapors of the volatile substance to be tested; (b) to provide for continuous flow of a stream of the vapor-air mixture at a constant rate through chambers containing living seedlings; and (c) to maintain constant environmental conditions for the desired period of time. Since no such apparatus has

been described in the literature, one especially designed to meet the above requirements was constructed for the present studies.

A diagram of this apparatus is shown in figure 1. Air was introduced

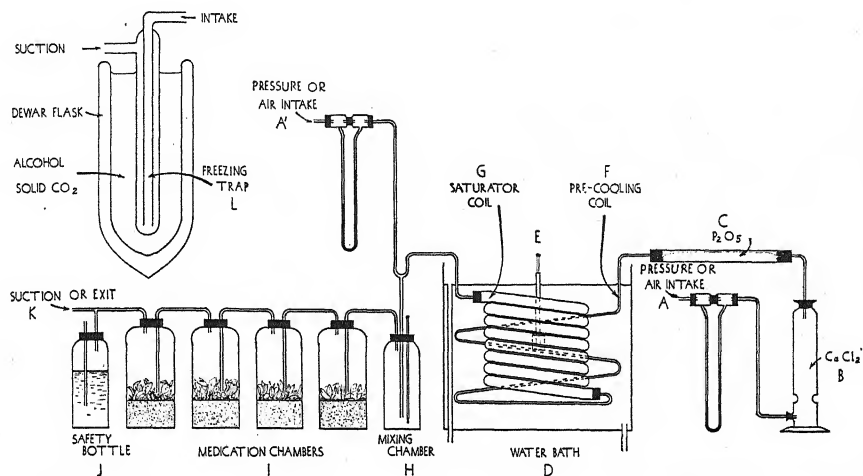


FIG. 1. Diagrammatic plan of apparatus employed to study toxicity of paradichlorobenzene.

into the apparatus through a calibrated flowmeter A. The air was partially dried by passage over calcium chloride B, and further desiccated over phosphorus pentoxide C, in order to prevent condensation of moisture in the saturator G and its subsequent accumulation in the absorption tubes or freezing traps L. In the experiments performed, using low concentrations of the vapor, the air was passed over concentrated sulphuric acid to assist in the removal of moisture. The dry air was then led into a copper tube of small bore coiled on the inside of the water bath D. This precooling coil F insured that air introduced into the saturator G would be of the proper temperature. Saturation of the incoming dry air was effected by leading it through a coil consisting of approximately 22 feet of $\frac{1}{2}$ in. copper tubing that had been filled loosely with crystals of PDB of a size corresponding to the manufacturer's grade No. 6.² The vapor-air mixture, water bath, and coil were maintained at constant temperature during the course of the experiments. The temperature of the bath D was regulated by means of ice or was kept constant by flowing tap water. A milled copper block was soldered to the saturator coil G and a drill hole, with tube, was used to bring a thermometer bulb E in close contact with the wall of the saturator G. It is preferable to use two thermometers, one at each end of the saturator coil G. The air saturated with vapor was next led into a short copper tube submerged in the bath in order to prevent heat transfer from the atmosphere to the outlet of the saturator. The opening of this tube led into a Y tube, one

² The paradichlorobenzene employed, 99.8 per cent pure, was kindly supplied by the Solvay Sales Corporation, New York, N. Y.

arm of which was connected with a calibrated flowmeter with air intake A'. The other outlet of the Y tube led to a mixing chamber H, and the gas was drawn over the plants in chambers I similar to those described in an earlier paper (5). These chambers could be placed in another water bath, having either the same temperature or a higher one than water bath D. Maintenance at lower temperatures than those of water bath D would result in precipitation of crystals from the vapor if saturation conditions should prevail. To move the gas through the system pressure is preferred to suction. A slight positive pressure is preferable to negative pressure, as it eliminates possible dilution of the vapor stream of fixed concentration by leakage of air into the system. All connections were of the butt-type and were carefully protected with shellac, since PDB vapor is appreciably soluble in rubber.

ESTIMATION OF PDB VAPOR CONCENTRATION

The concentration of PDB vapor in the gas-air mixture, delivered to the chambers containing tobacco seedlings, was estimated by freezing out the paradichlorobenzene from a known volume of the vapor-air mixture. This was done by permitting a measured volume of the mixture to pass through suitable freezing traps. These traps (Fig. 1, L) were constructed of thin pyrex tubing with outside dimensions of approximately 120 by 10 mm., the inner tube being 4 mm. in diameter. After stoppering the side arms with corks and weighing they were put into the system, replacing the plant chambers I. A calcium chloride guard tube was connected with the exit arm of the freezing trap to prevent entry of moisture. After sweeping the air and moisture from the freezing trap, the flow of vapor-air mixture was stopped to permit the freezing trap to be lowered into a Dewar flask containing a freezing mixture of 95 per cent ethyl alcohol and solid carbon dioxide. The flow was then resumed and a known volume was drawn through the trap at a fixed rate. The temperature of the freezing mixture and trap was maintained at approximately -70°C .

Since the vapor pressure of PDB at -70°C . is negligible, the weight (W) in grams per liter of PDB frozen from the dry vapor-air mixture becomes a direct quantitative measure of the PDB content of the mixture being drawn through the system. Since the weight of the vapor and the volume of the gas, together with the temperature and the total pressure, were known, the partial pressure of the vapor was computed from the gas formula.³

³ Where W is the weight of the vapor in g., R the gas constant in liter atmos., T the absolute temperature, M the molecular weight, V the total volume in liters, and p the partial pressure of the vapor in mm. then

$$p = \frac{WR}{MV} \times 760$$

From the partial pressure p , and the total pressure P both in mm., the volume per cent of vapor present is computed by the relation

$$\text{volume per cent} = \frac{p}{P} \times 100$$

Since the total pressure P varied but slightly from the normal atmospheric pressure, P was regarded as 760 mm. in these computations.

A measure of the accuracy of the method of analysis of the PDB content of the vapor-air mixture is shown by the series of measurements in table 1. In series 4, 5, and 6 the saturated vapor-air stream was diluted with 7.5 l. of air, whereas saturation was maintained in all others.

TABLE 1.—*Analysis of PDB content for vapor-air mixtures delivered by apparatus for determining its fungicidal value*

Series	Temperature of vaporization	Weight of PDB per gross volume of gas-air mixture	Partial pressure		Concentration of PDB
			Calculated from weight	Calculated from vapor pressure-curve	
<i>No.</i>	<i>°C.</i>	<i>G.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Vol. per cent</i>
1	12.5–13.0	0.0418 ^a	0.317	0.312	0.041
2	13.0	0.0439 ^a	0.332	0.320	0.043
3	13.0	0.0415 ^a	0.314	0.320	0.041
4	13.5–14.0	0.0246 ^a	0.187	0.163 ^c	0.024
5	14.0	0.0250 ^a	0.190	0.166 ^c	0.025
6	13.5–14.0	0.0247 ^a	0.188	0.163 ^c	0.024
7	0.2	0.0168 ^b	0.078	0.090	0.0103
8	0.2	0.0176 ^b	0.082	0.090	0.0108
9	0.2	0.0177 ^b	0.082	0.090	0.0108

^a 16 liters.

^b 24.82 liters.

^c Calculated from vapor pressure and dilution.

It becomes apparent by means of this method of analysis that a satisfactory approximation of the PDB content of a vapor-air mixture can be made. In this connection it may be pointed out that apparatus of the type just described should be generally useful in toximetric experimentation with other pathogens and with other volatile compounds.

DETERMINATION OF TOXIC AND FUNGICIDAL VALUE OF PDB

Methods

Following the development of apparatus capable of delivering a continuous stream of a vapor-air mixture at a constant PDB concentration, a series of experiments were undertaken to determine the maximal concentration of the fumigant tolerated by tobacco seedlings and the minimal concentration that is fungicidal. These experiments involved 1, varying the concentrations of PDB vapors and 2, varying the duration of exposure. The concentrations were varied by maintaining desired constant temperatures for saturating the air passing over the seedlings in the jars, or by diluting the vapor-saturated air mixture with a known volume of air introduced through the flow-meter A' (Fig. 1). After preliminary experiments involving exposures of 3, 6, or 9 hours, twelve-hour periods of exposure were selected. Applications were made at night. Each successive application was made after an interval of 12 hours.

The tobacco seedlings used included the varieties Yellow Mammoth, White Stem Orinoco, and Jamaica, all of which are equally susceptible to infection with downy mildew. They were grown in half-gallon, screw-cap,

glass jars containing approximately 500 g. of soil. When the seedlings were about 4 in. tall, the inoculum, consisting of a water suspension of freshly formed sporangia, was applied with a compressed-air atomizer. Conditions favorable for infection were then provided. Three or 4 days after inoculation the first application of PDB vapor was made, employing 3 to 5 jars of seedlings with each series. After each treatment the jars were aerated by directing a stream of air through the inlets until odor of PDB could no longer be detected. Absence of odor indicated that the concentration of PDB in the moisture present was less than 1 part per 100,000. This figure is approximately the lower limit of PDB concentration in aqueous solutions that gives a perceptible odor. It was estimated by the use of a Zeiss water interferometer to determine the amount of PDB in water solutions in which odor could just be detected. Experience showed that the jars required continuous aeration for several days to remove all traces of odor.

Two types of checks or controls were used, (a) inoculated nontreated seedlings and (b) noninoculated, nontreated seedlings. Fungicidal action following treatment with PDB was not apparent earlier than 6 to 8 days after inoculation, since this period corresponds with the length of the sporangial cycle. Toxicity to the host, however, was apparent either during the period of exposure or shortly thereafter.

The data resultant from these series involved seedlings in 183 jars, 131 of which were fumigated, while 42 served as inoculated controls and 10 served as healthy controls.

EXPERIMENTAL RESULTS

General Results

It was anticipated, as has been indicated, that the two factors (a) concentration of vapors and (b) duration of exposure to these vapors should be of primary importance, and, therefore, should be given major consideration in studying the toxicity of PDB. These factors would appear to be evaluated most succinctly by presenting certain selected data from among the body of data that has been secured. All other factors seem to be secondary, but it has not been possible quantitatively to assay the influence of each of these factors. Among the secondary factors that have been considered are mode by which tolerance of host and pathogen to PDB vapors is influenced by temperature and by presence of films of moisture on the foliage. Comparisons also have been made of tolerance of infected and healthy seedlings to PDB. It is clear from supplementary evidence that the above factors do not affect our results on the toxic limits within the precision of our determinations. Therefore, the following conclusions appear to be warranted. (a) The temperature at which seedlings are maintained during treatment, within the range 13° C. to 25° C. is without significant influence on the toxic and fungicidal limits. (b) The presence or absence of visible films of moisture on the foliage during fumigation does not appear to modify susceptibility of tobacco seedlings or of the pathogen to injury. (c) Infected

seedlings seem to be neither more nor less susceptible to injury than do healthy ones.

Influence of Varying Concentration of PDB

In contrast to the relative unimportance of the secondary variables just discussed is the influence of concentration and duration of exposure in determining toxic limits. The effect of concentration is clearly brought out by data in table 2, which apply to single applications.

TABLE 2.—*Fungicidal and toxic influence of varying concentrations of PDB. Single applications of 12 hours' duration*

Series No.	Total No. of jars used	Concentration of PDB vapors	Control of pathogen, No. positive or negative	Injury to host
		<i>Vol. per cent</i>		
1	11	0.01	Negative, 11	None
2	4	0.014	Negative, 4	None
3	4	0.017	Negative, 4	None
4	14	0.02	Positive, 12 Negative, 2	None
5	4	0.022	Positive, 2 Negative, 2	None
6	3	0.0375	Positive, 3	Slight
7	7	0.042	Positive, 7	Slight, 6 Severe, 1

The data in table 2 indicate that when single fumigations of 12 hours duration are given, there is a range of concentration of PDB vapors within which fungicidal action against tobacco downy mildew does not occur. With increased concentration, however, there is a range in which sporangial formation is inhibited without evidence of injury to the tobacco seedlings. As the volume-percentage concentrations are further increased the seedlings are injured, least damage occurring with the lower concentrations. The termini of these ranges are not sharply delimited, an observation entirely in accord with similar studies involving biological materials. PDB is slightly fungicidal if infected plants are exposed for a 12-hour period to an atmosphere saturated with PDB vapors within the range of 0.01 to 0.022 volume percentage (equivalent to saturation at 0° C. to 7° C.), but the pathogen is not eradicated. Eradicant action was exhibited at complete saturation within the range 0.022 to 0.0375 volume percentage, equivalent to saturation at 7° C. to 12° C. Definite injury to seedlings resulted if the concentration was above 0.037 volume percentage. This is equivalent to saturation at 12° C.

Influence of Repeated Applications

It seemed probable that volume-percentage concentrations within the range found to be nonfungicidal with a single application of PDB might be effective if applications were repeated on successive nights. The importance of repeated applications is clearly evident from the data in table 3.

As was anticipated, repeated applications of PDB were found to be more

TABLE 3.—*Fungicidal and toxic influence of repeated applications of PDB at varying concentrations*

Series No.	Total No. of jars used	Duration of fumigation	Concentration of PDB vapors	Control of pathogen, No. positive or negative	Injury to host
		<i>Hr.</i>	<i>Vol. per cent</i>		
1	8	12	0.01	Positive, 2 Negative, 6	None
2	8	24	0.01	Positive, 6 Negative, 2	None
3	8	36	0.01	Positive, 6 Negative, 2	None
4	8	48	0.01	Positive, 8 Negative, 0	None
5	4	12	0.02	Positive, 2 Negative, 2	None
6	4	24	0.02	Positive, 4 Negative, 0	None
7	4	36	0.02	Positive, 3 Negative, 1	None
8	4	48	0.02	Positive, 4 Negative, 0	None
9	4	12	0.022	Positive, 2 Negative, 2	None
10	5	24	0.022	Positive, 5 Negative, 0	None
11	6	36	0.022	Positive, 6 Negative, 0	None
12	5	36	0.0467	Positive, 5 Negative, 0	Severe
13	5	48	0.0467	Positive, 5 Negative, 0	Severe

effective than a single application. The data indicate that within the range 0.01 to 0.02 volume-percentage concentrations, effective fungicidal action follows the use of 4 applications on consecutive nights. Three consecutive fumigations were required at a concentration of 0.022 and caused no injury to the plants. Concentrations of 0.046 used on 3 or 4 successive nights, however, caused severe injury to the seedlings.

GENERAL CONSIDERATIONS

The experimental results with PDB illustrate the principles that have already been set forth concerning the mode of action of volatile fungicides (8). An appreciation of the mode of action of volatile fungicides may be had if certain fundamental physical and chemical facts are borne in mind. Volatile chemicals are distributed over both the external and the internal surfaces of the leaves by virtue of their ability to evaporate at ordinary temperatures. By virtue of their solubility in water they enter into solution in external aqueous films, and in moisture within the cell where they may react with cell constituents. The concentration of vapors in water is determined (a) by the partial vapor pressure of the volatile chemical in the atmosphere in contact with aqueous films, and (b) by temperature. Fungicidal action depends upon the two factors, volume-percentage concentration and duration of treatment. While the fungicidal value of any given volatile

compound is related to each of the factors enumerated, there is also a specific effect. This depends on the differential interaction of the compound with host and pathogen, respectively. It may be either physical, involving effects such as solvent or surface action, or it may be a specific chemical interaction with particular cell constituents.

As concerns PDB there is a relationship between temperature, vapor pressure, volume-percentage concentration and the ranges within which injury to tobacco seedlings and to *Peronospora tabacina* occurs. This can best be appreciated if presented graphically, as in figure 2. This graph is based on findings in the present study and on PDB vapor-pressure measurements made in our laboratories and reported elsewhere (2). In it vapor pressure and the corresponding saturation vapor concentration are plotted as functions of the temperature. The limitation imposed by temperature

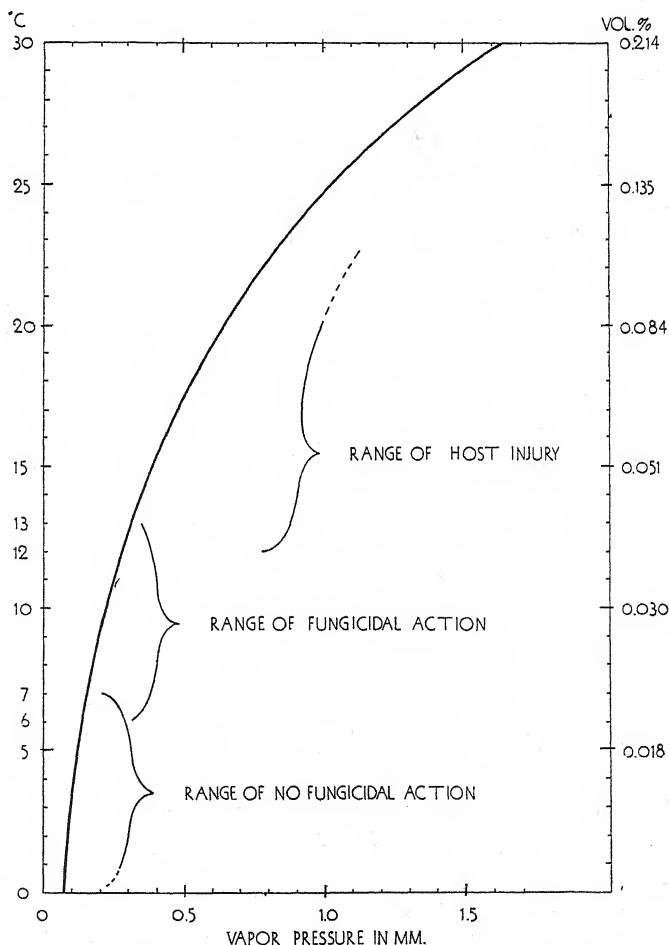


FIG. 2. Graph showing relationship of temperature, vapor pressure and volume percentage concentration of paradichlorobenzene at saturation and their ranges of toxic action in one 12-hour period.

on the vaporization of PDB is at once apparent. In the lower ranges the vapor pressures and, therefore, the saturation concentrations increase very slowly with temperature.

PRACTICAL IMPLICATIONS

While it has been possible by repeated fumigation to control tobacco downy mildew under laboratory conditions with saturation vapor pressures of PDB within the temperature range 0° to 7° C., this compound might be ineffectual in seedbeds within this temperature range for the reason that it would be impossible, because of leakage, to secure and maintain these saturation pressures. This limitation in use of PDB as a fumigant against tobacco downy mildew in seedbeds may be circumvented by certain procedures, as will be considered in an accompanying report (4) that details the results of field experiments.

HOST-PENETRANT FUMIGANTS

In view of the novel mode of action of PDB and related volatile substances, it seems desirable to designate them as host-penetrant fumigants. This emphasizes the important distinction that arises because of their ability to penetrate within tissues and act therein. This is in contrast to the immobility and lack of host-penetrating power of such agents as sprays and dusts that function as protectants on the external leaf surfaces.

It is clear that these penetrants could act in a number of different ways. In the cases of benzol and of PDB this action, which, either directly or indirectly, involves the host or the pathogen, is so effective that these compounds act as eradicants. The importance of such penetrant action is apparent when it is realized that it is possible by means of volatile fungicides to check the course of infection. In this sense they may be said to serve as curatives.

SUMMARY

Paradichlorobenzene, a volatile crystalline compound, is fungicidal to *Peronospora tabacina* and acts as an eradicant without appreciable injury to the host.

The minimal concentration of paradichlorobenzene vapor fungicidal to tobacco downy mildew is within the range 0.01 and 0.02 volume percentage, equivalent to saturation pressures within the temperature range 0° C. to 7° C. Three to 4 consecutive treatments within this range are requisite for effective fungicidal action. A single application within this range does not effect eradication.

The maximal concentration of paradichlorobenzene vapor tolerated by tobacco seedlings for a single 12-hour fumigation is approximately 0.0375 volume percentage, equivalent to saturation at 12° C.

Temperature also is a factor of major importance in delimiting the concentration of PDB vapor obtainable.

The toxic limits of PDB were determined by an apparatus that should be generally useful in toximetric experimentation.

The concept of penetrant fumigants is developed and its implications are indicated.

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THE USE OF PARADICHLOROBENZENE IN SEEDBEDS TO CONTROL TOBACCO DOWNY MILDEW^{1,2}

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INTRODUCTION

Field experiments involving the employment of paradichlorobenzene ($p - C_6H_4Cl_2$) as a fungicide for preventing and controlling downy mildew on tobacco seedlings are here reported. In planning these experiments use was made of the results of field studies, performed in 1938, and of laboratory studies (2) performed during the current season. Only a limited number of field experiments with this volatile crystalline product had been performed prior to the current year. The laboratory studies have dealt with such fundamental problems as rate of vaporization in relation to size of crystals and to temperature, with methods for analysis of paradichlorobenzene in vapor-air mixtures, with measurements of the vapor pressure of this compound (1, 2), and with determinations of the minimal concentration of vapor that is fungicidal to the downy mildew fungus, and that which is toxic to tobacco seedlings. As might be anticipated, previous experiences with benzol as a fungicide were found valuable in the present studies.

The purpose of the present work was (A) to determine whether paradichlorobenzene (for brevity, hereafter designated as PDB) constitutes a de-

¹ Cooperative investigations conducted by the Virginia Agricultural Experiment Station and Duke University.

² Special thanks are due Mr. E. G. Moss, Tobacco Experiment Station, Oxford, N. C., for his whole-hearted cooperation.

pendable fungicide against tobacco downy mildew, and (B) to evaluate the modificatory influence of certain mechanical and environmental factors as (a) PDB vapor concentration within seedbeds, (b) temperature, (c) moisture conditions, (d) distribution of crystals, (e) amount of fumigant, (f) tightness of seedbed frames and covers, (g) volume of seedbed, (h) size of crystals and (i) interval between successive applications. A knowledge of these interrelated factors should be of value in seedbed practices. Not all of them, however, have been isolated and evaluated, but a body of experimental evidence applying to the use of PDB has been secured that contributes to an understanding of this problem.

METHODS AND MATERIALS

Three locations were selected in which to conduct these experiments, one near McDonald, North Carolina, representative of a poorly drained area of the lower Coastal Plain; another near Oxford, North Carolina, representative of the upper Coastal Plain; and the third near Chatham, Virginia, representative of the Piedmont area. By taking advantage of seasonal differences existing in these 3 localities, the duration of the period for making observations was extended over more than 2 months.

Weather conditions during 1939 were considered only moderately favorable for the development of the disease in each of the selected localities. Sporulation was first observed in the experimental seedbeds near McDonald on March 24, near Oxford on April 15, and near Chatham on April 28. Within each locality the severity of downy mildew varied greatly, depending upon soil-moisture conditions, air movement, and proximity to woods.

The seedbeds were framed with boards and divided into convenient compartments. Some of the beds were made by growers, and were either not framed or framed with logs. The covers were made to fit tightly, and, except in 2 series of tests involving the influence of covers of different textures, consisted of unbleached sheeting having 64 warp threads and 64 woof threads per inch, 3.5 sq. yd. of which were required to weigh one pound.

A technical grade of PDB, 99.8 per cent pure, was used.³ The crystals of PDB were distributed either on shelves constructed of boards or on wire screens arranged along the sides near the top of the framing. Alternatively the crystals were broadcast over the top of the ordinary loose-texture seedbed cover (Fig. 1, A). The denser heavier covers were, of course, drawn over the ordinary cover after the crystals had been distributed.

Applications were made between 6 and 7 o'clock p. m., and the heavy covers were removed approximately 12 hours later.

Sampling the atmosphere within the seedbeds to determine its PDB vapor content was accomplished by means of a specially constructed aspirator (Fig. 1, B). This necessitated the installation of copper tubes at selected positions so that the inlet was inside the bed and the aspirator could be attached to the outlet. Aspiration was effected by displacement of water.

³ The sizes of the crystals employed were those designated by the manufacturers, The Solvay Sales Corporation, as grades No. 1, 6, and 9.

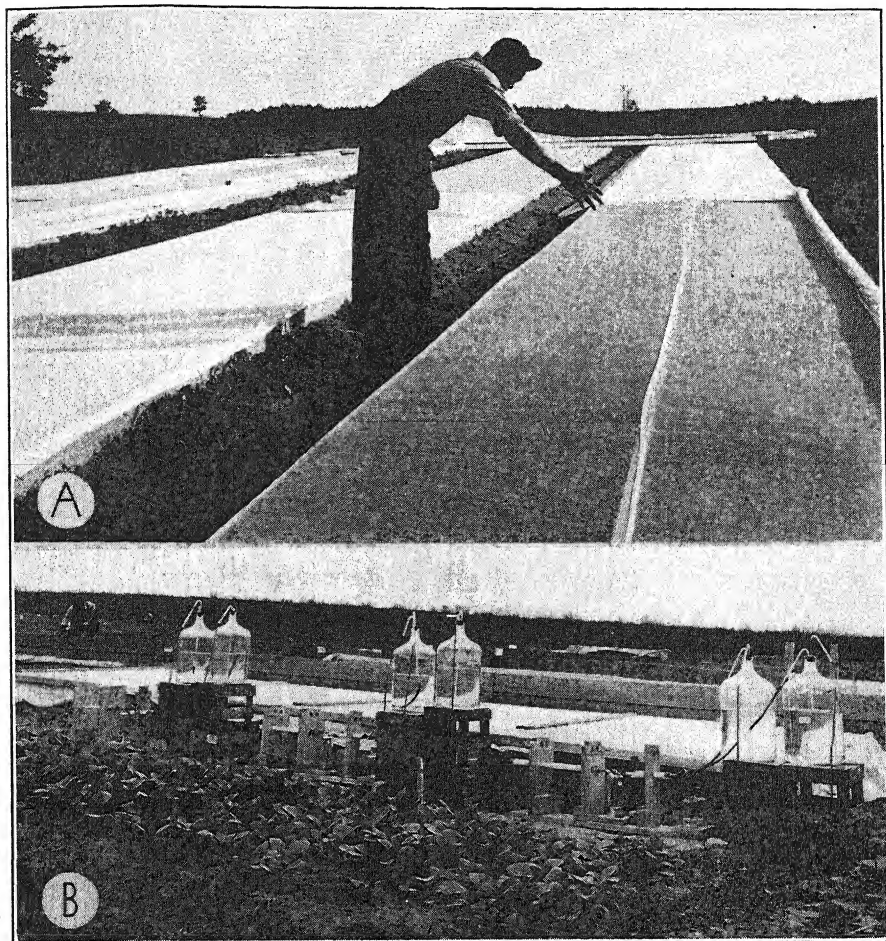


FIG. 1. A. Method of broadcasting crystals of paradichlorobenzene on ordinary seed-bed cover. The heavy cover appears rolled back on bed in foreground. B. Aspirators used in sampling vapor-air mixtures in seedbeds.

The vapor-air mixtures were partly dried by passage over NaOH flakes. The PDB was removed from the vapor-air mixture by freezing traps, as previously described (2). From the weight of PDB crystals in the traps, the volume-percentage concentration of vapors in the atmosphere of the seedbeds was calculated (2).

EXPERIMENTAL RESULTS

Prevention and Control of Downy Mildew in Seedbeds

Tests of the efficacy of PDB were designed to determine whether this compound can be employed both to prevent and to control downy mildew in each of the 3 selected localities. Preventive applications were begun prior to the appearance of disease in the experimental beds, while applica-

tions to control were begun immediately after sporulation first became evident. Forty-seven seedbeds, ranging in size from 4 to 200 sq. yd. and having a total area of 1720 sq. yd., were employed. The treatments were successful in 36 of these beds and failed in the remaining 11 beds. Treatments were regarded as successful if further sporangial formation was completely inhibited or if sporangia formed on only 1 or 2 leaves throughout the entire period of treatment. The total area of successfully treated beds was 1612 sq. yd., and of unsuccessfully treated areas, 108 sq. yd. In the case of the successful treatments, PDB was applied every night in beds having a total area of 372 sq. yd.; and a total area of 1240 sq. yd. of seedbeds was treated at successive intervals greater than one night. Of the beds in which PDB failed to give satisfactory control 80 sq. yd. were treated every night and 28 sq. yd. were treated at successive intervals greater than one night. Consideration will be given subsequently to factors that determine whether treatments with PDB will successfully prevent or control downy mildew of tobacco or fail to do so. Suffice it to conclude that successful control was accomplished in each of 3 localities during a season when the disease was moderately severe, both by nightly application of PDB and by employing longer intervals between successive applications.

MODIFYING FACTORS

A proper understanding and interpretation of the results of experimentation with PDB as a fungicide against tobacco downy mildew necessitated that account be taken of the influence of several obvious factors. Primarily, these included the effect of the application of varying quantities of the chemical, and the effects of moisture and temperature conditions, in so far as they modify the evaporation of the crystals and the absorption of the fungicidal vapors. Consideration was given also to certain other factors that, although important, appear to be secondary. These include the mechanics for best distribution of crystals to insure the most efficient fungicidal action, variation in the frequency of applications, and the influence of size of crystals and of porosity of covers. In pointing out the influence of any single factor, in the accounts that follow, detailed statements concerning all other factors are omitted in order to avoid repetition. Such factors were either identical or were kept as nearly constant as is possible in experimentation involving seedbeds.

In a previous study (3) use was made of measurements of the volume-percentage concentrations of the benzol vapors present within the seedbed atmosphere throughout the night to evaluate certain factors governing the effectiveness of benzol, applied to tobacco seedbeds, in controlling downy mildew. Measurements were, therefore, instituted in the present studies with PDB to aid in evaluating the factors that contribute to proper use of this fungicide. Since measurements of volume-percentage concentrations of PDB in vapor-air mixtures by means of freezing traps are both arduous and time consuming, only a limited number were made.

Influence of Varying Quantities of PDB

In this series of experiments measurements were made of concentrations of PDB vapors in the atmosphere of three seedbeds that were similar in every other respect, except that the equivalent of 1, 2, and 3 pounds of fumigant respectively per 100 sq. yd. of seedbed area was distributed over the surface of the tobacco seedbed covers. The heavier covers were then placed over them. The night remained continuously warm and there was no appreciable formation of dew. The essential conditions of this experiment and the results of the measurements are assembled in table 1.

TABLE 1.—*Influence of the application of varying quantities of PDB on the concentration of vapors in the air of seedbeds*

Amount of PDB applied per 100 sq. yd.	Temper- ature	Time elapsed after appli- cation	Concentration of PDB vapors in atmosphere	Moisture conditions
<i>Lb.</i>	<i>°C.</i>	<i>Hr.</i>	<i>Vol. per cent</i>	
1	16	5	.0025	Covers and foliage dry
1	15	8	.0009	do
1	17	11½	.0013	do
2	16	5	.0037	do
2	15	8	.0018	do
2	17	11½	.0014	do
3	16	5	.0046	do
3	15	8	.0025	do
3	17	11½	.0016	do

These data show that as the quantity of crystals applied per unit area of seedbed increases, the volume-percentage concentration of the vapor in the atmosphere also increases.

Influence of Moisture

Comparative measurements of volume-percentage vapor concentrations of PDB in the atmosphere of seedbeds were made under identical conditions, except that the seedlings and the covers were sprinkled to keep them thoroughly wet in one case; in the other, the foliage and covers remained rather dry. Representative contrasting measurements of the vapor concentrations found are shown in table 2.

As was anticipated, markedly higher concentrations of vapors occur in

TABLE 2.—*Influence of moisture conditions upon concentration of vapors of PDB in the atmosphere of seedbeds*

Amount of PDB applied per 100 sq. yd.	Temper- ature	Time-elapsed after apply- ing PDB	Concentration of PDB vapors in atmosphere	Moisture conditions
<i>Lb.</i>	<i>°C.</i>	<i>Hr.</i>	<i>Vol. per cent</i>	
4.0	14.4	3.0	.0094	Foliage and covers dry
4.0	14.0	5.5	.0094	Foliage and covers dry
4.0	14.4	3.0	.0170	Foliage and covers wet
4.0	14.0	5.5	.0170	Foliage and covers wet

seedbeds when covers and foliage remain wet throughout the night. Evidently, the covers greatly retard loss of PDB vapors if they are wet, whereas they are much less effective in this respect if kept dry. This observation accords with previous findings relative to the influence of moisture on the covers in retarding the escape of benzol vapors (3).

Influence of Temperature

In data previously presented (2) the relationship between temperature and vapor pressure of PDB is clearly shown. From these data one would anticipate finding that differences in volume-percentage concentration of vapors should be correlated with differences in temperature. Measurements of vapor concentrations involving 2 contrasting temperature conditions are shown by the 2 following cases. In the first instance the equivalent of 3 pounds of crystals per 100 sq. yd. of seedbed area was applied in the daytime to the selected seedbed. The crystals were scattered upon the surface of the ordinary cloth, the heavy cover was drawn into position and wetted. Two hours after fumigation was begun, the temperature within the seedbed being 30° C., the volume-percentage concentration of PDB vapors was 0.0114. In another bed similarly treated, but in which the temperature was 19.5° C., the volume percentage concentration of PDB vapor was 0.0039, when measured 3 hours after fumigation was begun.

The influence of temperature also was approximated by collecting and weighing the crystals that remained in the morning. On nights when temperatures were 7° C. or lower, two-thirds of the crystals remained unvaporized in beds where the equivalent of 1.5 lb. of fumigant per 100 sq. yd. was used.

Insufficient vaporization occasioned by low temperature may result in failure to control tobacco downy mildew, under the limitations imposed by application in seedbeds. Such a condition was encountered during the past season in a cold, rainy period extending from the late afternoon of May 1 to mid-forenoon of May 4. The temperature dropped suddenly, beginning about 6 p. m. on May 1, at which time 4 lb. of PDB crystals were applied to a seedbed of 110 sq. yd. Within 3 hours the temperature had fallen to below 50° F. and, thereafter, remained continuously between 50° F. and 40° F. for 36 hours. Limited evaporation of crystals occurred during the first night and the heavy cover was allowed to remain undisturbed throughout the following day, since it was continuously cold and rainy. Sporulation was abundant on the morning of both May 2 and 3. A portion of the crystals still remained unvaporized on the latter morning when the bed was uncovered. The temperature during the day of May 3 rose to between 50° and 60° F., but the sky remained overcast. Another application of 4 lb. of PDB crystals was made during that afternoon. The temperature again fell to below 50° F. by 8:00 p. m., and gradually became colder throughout the night. Sporulation was again profuse on the morning of May 4, when it was found that an abundance of the PDB crystals still remained unvapor-

ized. Control in this instance was obtained by allowing the cover to remain undisturbed during the forenoon of May 4. The day was warm and sunny so the rise in temperature increased vaporization.

Influence of Distance from Source of PDB

PDB vapors are approximately 5 times heavier than air and, therefore, they could be expected to diffuse slowly throughout seedbeds, since the rate of diffusion of a vapor is inversely proportional to its density. The data in table 3 indicate the differences that can exist in distribution of PDB vapors as related to distance from source.

TABLE 3.—*Influence of distance from source of PDB upon concentration of vapors within the air of seedbeds*

Amount of PDB applied per 100 sq. yd.	Temper- ature	Time elapsed after appli- cation	Concentration of PDB vapors in atmosphere	Source of samples
<i>Lb.</i>	<i>°C.</i>	<i>Hr.</i>	<i>Vol. per cent</i>	
3.0	13.0	3	.0055	Sampled near source
3.0	10.4	6	.0092	do
3.0	10.0	8	.0077	do
3.0	10.0	10	.0081	do
3.0	13.0	3	.0020	Sampled at soil surface
3.0	10.4	6	.0031	do
3.0	10.0	8	.0035	do
3.0	10.0	10	.0034	do

The greater concentration of vapors may be noted to occur in the air near the crystals. Probably other factors, as absorption by moisture on the foliage and in the soil, were of more importance in determining the distribution of vapors within the bed than diffusion.

Amount of Paradichlorobenzene

Evidence from experience of the previous year had indicated that PDB, applied nightly at the rate of 1.5 lb. per treatment per seedbed area of 100 sq. yd., is sufficient to be effective in the control of tobacco downy mildew. In the present experiments the quantities applied were equivalent to 1, 1.5, 2, 3, or 4 lb. nightly.

In certain series, fumigation was initiated before outbreak of downy mildew; in others, treatments were applied only after infection was apparent. In any case satisfactory control resulted from the use of each of these quantities of PDB, under each set of conditions, when the fumigant was broadcast. Injury to the seedlings, manifest in yellowing of foliage and bleaching of tips of the older leaves, was apparent in beds that received the larger amounts. Most pronounced injury occurred if the temperature at night ranged from 20° to 25° C. In no case did injury to the host result from the use of 1 or 1.5 lb. per seedbed area of 100 sq. yd.

Size of Paradichlorobenzene Crystals

A study of comparative rates of evaporation of crystals of different sizes shows that the rate increased with decrease in size of crystals (1). Attempts

to take advantage of the slow rate of evaporation of large crystals (No. 1 size) to maintain low vapor concentrations in the beds, both night and day, were unsuccessful. In subsequent experimentation only smaller crystals of sizes 6 and 9 were employed in order to obtain as complete and as rapid vaporization as possible during the time available for each treatment. Both of these sizes, under comparable conditions, gave satisfactory control. Crystals of size 6 are preferred, however, because those of size 9 tend to cake, when applied on shelves, and to sift through when applied on the top of seedbed covers.

Distribution of Paradichlorobenzene

As has previously been stated, 2 methods were employed to distribute the crystals of PDB. One method involved shelves, either of boards or of screen-wire, arranged inside the bed along 2 sides, along 1 side, along the center, or some other modification of this arrangement. The other method involved broadcasting the crystals over the top of the thin cover in order that the area of distribution might equal that of the seedbed. Although satisfactory control followed the use of each method, failures resulted in some cases when the crystals were scattered on shelves. These failures appeared to be related to placement, distribution, and area of the shelves; but data are insufficient to show what arrangement of shelves would be satisfactory under all conditions. The best procedure appears to be to scatter the crystals over the top of the ordinary seedbed cloth and then cover this over with the heavier cloth.

Tightness of Seedbeds

Type of seedbed cover is an important consideration when employing volatile materials in the control of downy mildew. It may be recalled that there was lack of accord among the results of experiments performed in 1938, as to the type of seedbed cover required for successful fumigation with PDB. Accordingly, 1.5 lb. of crystals per application of 100 sq. yd. of seedbed area were employed, using 5 types of cloth with specifications as follows: (A) warp 24, woof 28, 14 sq. yd. to weigh 1 lb.; (B) warp 48, woof 48, 7 sq. yd. to weigh 1 lb.; (C) warp 48, woof 48, 4 sq. yd. to weigh 1 lb.; (D) warp 56, woof 60, 4 sq. yd. to weigh 1 lb.; (E) warp 64, woof 64, 3.5 sq. yd. to weigh 1 lb. The first and second types of cover correspond closely in density of weave to open-mesh and close-mesh tobacco-seedbed covers of the kinds ordinarily employed on seedbeds. They were given a careful trial because of statements to the effect that control of downy mildew could be obtained from the use of PDB, even with the ordinary seedbed covers. No apparent control resulted with the use of the first type of cloth in either of the two beds, the one treated every night, the other on alternate nights. Complete lack of control occurred on each bed, treated every night, when covered with the second type of cloth. Control was entirely satisfactory in each of the beds covered with the other 3 types of cloth. These results indicate that the covers must be sufficiently densely woven to become vapor-tight when

moistened, and that loosely woven covers possess little or no ability to retain vapors of PDB within the beds.

Type of framing constitutes a second consideration in making seedbeds sufficiently tight for effective fumigation. Boards can be properly joined to insure tightness. It is more difficult to make the seedbeds tight if log frames are used, but such beds, 200 sq. yd. in area, have been successfully treated when the crystals were applied upon the thin cover and if the heavy cover drawn over the top is sufficiently large to permit the lap to be firmly secured close to the ground outside of the frame.

Volume of the Seedbed

Emphasis has hitherto been placed upon surface area of the seedbed in determining the quantity of fumigant to apply. Manifestly, total volume as affected by depth of the seedbed is also a factor that should be taken into consideration. Our experiments have not involved the comparison of beds with frames of different heights. The depth of the beds used has been quite uniform throughout, and since the heavy covers were normally fitted tightly over the frames, the depth of the beds corresponded with the height of the frames. In certain cases however, the volume was decreased by allowing the heavy cover to sag down in the center of the bed. As a result of this decrease in volume the PDB vapor concentration within the bed was increased, as was anticipated. These observations suggest that fungicidal activity may be modified, if all other factors are constant, by elevating or lowering the heavy cover, and thus varying the volume of the seedbed.

Interval between Treatments

The apparent eradicator action of PDB suggested that fumigation might be effective if the interval between successive applications was longer than 1 night. Accordingly, experiments involving seedbeds at Oxford and Chatham were planned and treatments were made on alternate nights, at intervals of 3 nights, and at intervals of 4 nights between successive applications. The PDB was applied in amounts of 3.0 and 4.1 lb. per application per area of 100 sq. yd. Injury to the seedlings resulted from the use of the larger amount, whereas satisfactory control without injury to the seedlings followed the use of 3 lb. applied on alternate nights or at successive intervals of 3 nights. Satisfactory control resulted from treatments made every fourth night in some of the trials, provided the covers were thoroughly wetted.

When it became apparent that PDB may act as an eradicator fungicide and may be used successfully to control downy mildew, either by nightly applications or according to a fixed schedule in which the interval between applications is longer, attempts were made to test the effectiveness of applications made at irregular times, as indicated by best judgment. Only infected seedbeds were used in these experiments. Several factors were taken into account in judging when to treat and in approximating the amount to

apply. The severity of the disease and weather conditions were of primary importance in judging when to apply PDB. Some of the treatments were made in the daytime to take advantage of the influence of increased temperature on the rate of vaporization of the crystals. This factor, together with the volume of the seedbed, was taken into account in judging the quantity of fumigant to apply. In all cases the crystals were broadcast upon the thin covers just prior to fitting the heavier covers over the beds. A total of 1364 sq. yd. of seedbed was treated in this series of experiments, with the result that satisfactory control was accomplished in all. However, since the season was not an extremely severe one with respect to incidence of the disease, further experience with the above procedure involving applications at irregular intervals should be obtained before it is generally recommended as applicable to all conditions of downy-mildew incidence.

GENERAL CONSIDERATIONS

The concentration of PDB vapor in water that inhibits germination of sporangia of *Peronospora tabacina* (4) has been found to be of the same order of magnitude as that that is fungicidal, as determined by laboratory studies (2) using infected plants maintained in an atmosphere having a constant volume-percentage PDB vapor concentration. All of the measurements of volume-percentage vapor-concentration in the atmosphere of seedbeds, however, as shown in tables 1, 2, and 3 yield a lower figure. The explanation of this apparent discrepancy lies in differences in conditions existing between laboratory and seedbed experiments. In the laboratory, infected seedlings were maintained at a constant temperature and a constant concentration of vapors for periods of 12 hours, so that a condition was reached wherein the PDB content of the air was in approximate equilibrium with that of the plant tissues. Such an equilibrium condition is, no doubt, never approximated in seedbeds. The vapors of PDB, however, appear to be extensively absorbed by the plants, the soil, and the wet covers; the soil, in particular, serves as a reservoir for storage of dissolved PDB. The fumigant reevaporates from the soil when the atmosphere in the bed loses its vapor content through leakage or otherwise.

The persistence of the odor of PDB in the atmosphere of seedbeds until late in the morning after the covers are removed may be regarded as evidence of absorption and reevaporation of PDB vapors by the soil and the seedlings.

The low vapor pressure of PDB at the lower temperatures and its relative insolubility indicate that this fumigant might not be equally effective in all seasons against tobacco downy mildew in seedbeds. While the results of our laboratory experiments (2) show that the pathogen yields to control if two or three applications of 12 hours each are made on successive nights with concentrations of PDB corresponding with saturation at 0° C., it is difficult if not impossible to approach such saturation concentrations at the same temperatures in the seedbeds. In case extended periods prevail during

which the temperatures remain below 7° C., the use of PDB might not control, as has previously been pointed out in this report in experiments involving the influence of temperature.

Manifestly, the employment of any measures during cold weather, which would increase the vaporization of PDB and would minimize the loss of vapors from the bed, would make it more nearly possible to approach concentrations that are effective. In cold weather the use of larger quantities of properly distributed PDB crystals would increase the area of the crystal surface whence vaporization could take place, and, therefore, would tend to build up higher vapor concentrations within the bed. Of course, much of this larger amount of crystals would be found, in the morning, to have remained unvaporized.

Even though the periods of cold weather lasted 3 or 4 days, it might still be possible to control tobacco downy mildew by the use of PDB. This could be accomplished (a) provided the pathogen had been eradicated within the beds prior to the advent of the period of cold weather, or if, by fumigation, the seedlings had been completely protected against infection before the cold weather began, and (b) provided the duration of the cold period was less than the length of the sporangial cycle, *i.e.*, 5 to 7 days. Control might also be accomplished by allowing the heavy covers to remain in place in the morning until the seedbeds have been warmed by the sun to the point where fungicidal concentrations of PDB vapors had been built up. The length of time that covers may be permitted to remain on the beds in the morning must be carefully limited, because experience has shown that concentrations toxic to the seedlings may develop if the covers are not removed after 2 or 3 hours of sunshine.

In spite of these limitations imposed by cold weather, the results of the past 2 seasons have abundantly shown that, under ordinary weather conditions, PDB can be applied as an effective control of tobacco downy mildew. The application of the results of these field studies to seedbed practices indicates that the successful use of PDB as a fumigant requires an appreciation of the interdependence especially (a) of temperature conditions, (b) of tightness of seedbeds as affected by construction of frames and by moisture on the covers, (c) of quantity of the fumigant applied per unit area of seedbed, and (d) of proper distribution of the crystals to be vaporized.

SUMMARY

Paradichlorobenzene has given satisfactory control of downy mildew of tobacco in seedbeds, either when applied on successive nights or over longer intervals between successive applications.

This volatile compound may be regarded as an effective fungicide against tobacco downy mildew when employed under the following conditions: (A) The proper amount of paradichlorobenzene is from 1.5 to 3 lb. per application per seedbed area of 100 sq. yd. (B) The crystals should be distributed widely on the top of the loose-texture cloth ordinarily used for seedbed

covers. Sheetting of approximately 60 threads each way per inch should be used as a covering during fumigation. (C) Under seedbed conditions temperatures above 7° C. are necessary for vaporization to proceed sufficiently to maintain effective vapor concentrations. (D) Moisture on the covers is desirable as an aid in retaining effective concentrations of vapor within seedbeds.

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NEW STAGES OF SPOROCYBE AZALEAE

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(Accepted for publication Jan. 30, 1940)

Sporocybe azaleae (Peck) Sacc. causes a bud and twig blight of azaleas and rhododendrons. This fungus is imperfectly known but is generally recognized by coremia that appear on the infected flower buds, the leaf and stem buds, leaf scars, old stems, floral parts, and on the fruits.¹

Peck,² in 1873, named this fungus *Periconia azaleae*, but Saccardo,³ in 1886, transferred the genus to *Sporocybe*; since then it has been known as *S. azaleae*. This Latin binomial refers to the "spore-heads" of the fungus heretofore described. Relying on this description, mycologists have placed the fungus in the taxonomic keys adjacent to *Graphium ulmi* Schw., cause of the Dutch elm disease.

The separation of these 2 genera is based on color of the coremia and spores. Those with light-colored coremiospores are *Graphium*; those with dark-colored ones are *Sporocybe*.

Graphium ulmi has 3 spore stages or forms listed; a coremial stage assigned to *Graphium*; a conidial stage assigned to *Cephalosporium* and an ascogenous stage assigned to *Ceratostomella*. *Sporocybe azaleae*, however, has only the coremial stage listed. Since these 2 fungi are taxonomically related, one might expect them to possess similar stages in their life histories.

¹ Description of the disease, pathological anatomy, methods of culturing the fungus, together with inoculations of hosts, have been described in *Phytopath.* 29: 517-529. 1939.

² Peck, C. H. *Periconia azaleae*. New York State Museum of Natural History Ann. Rpt. 25: 93. 1873.

³ Saccardo, P. A. *Sylloge Fungorum* 4: 608, 1886; 10: 692-693, 1892; 11: 643-644, 1895; 12: 744, 1897; 20: 861, 1911.

An investigation was undertaken in 1930 to test this supposition by culturing the fungus.

Monosporous cultures were prepared by isolating coremiospores (Plate I, I) removed from azalea buds and germinated in van Tieghem cells. From these cultures mycelium was implanted on potato-dextrose agar and incubated at 22° C.

HYPHAE, MYCELIUM, AND SCLEROTIA

Young hyphae at the margin of a maturing colony, incubated on potato-dextrose agar for 1 week, appeared either gray or nearly hyaline (Fig. 1, F and Plate I, A). The average weekly growth of 6 of these cultures incubated at 22° C. was 4 mm. radially and 2.7 mm. vertically. However, when maturing, they changed from gray to either a citrate drab or to a deep gray-olive, but sporulating hyphae were often a snuff-brown. The cells in vegetative hyphae growing on artificial media averaged $4 \times 8 \mu$, but, when growing in host tissues, $3 \times 5 \mu$. In a sclerotium some of the cells were rectangular, $1.7 \times 3 \mu$, while others emerging were much larger in diameter. One such hypha was 6μ in diameter and consisted of cells 54, 72, 18 and 58μ in length. Cells in sporulating hyphae that formed conidiophores averaged $1.8-2.7 \times 4-29 \mu$ (Fig. 1, D).

The cell walls of old hyphae located at the center of mature cultures were either a snuff-brown or a warm-sepia. When located in the substratum and viewed by direct light, they were brownish but, by reflected light, a blue purple undercolor which gave the culture a distinguishing characteristic.

Old hyphae often are constricted at their cross walls; the branching is sparse and forms angles of 80° to 90° (Fig. 1, F; Plate I, H). In cultures the first branching was noted after an incubation of 72 hours, when the germ tubes averaged 300μ in length.

Hyphae sometimes entered host cells and formed "skeins" and "knots" which are here considered haustoria. In no case were woody vessels observed to be plugged by these hyphal knots.

On water agar, the hyphae that gave rise to conidiophores remained a light-gray and sparsely septate. They quickly permeated the agar, leaving behind only a sparse surface growth to form cephalospores. On nutrient agar, however, the hyphae were dark-gray or brown, slower to penetrate the substratum, their cells larger, and they formed a definite mycelial mat or subicle, with an abundance of spores. Thus it is to be noted that the nature of the substratum or agar varied the size, shape, color, and amount of hyphae.

Sometimes cultures formed pionnotes, especially when produced through transfer from old cultures that had been incubated for 6 months. This type of culture sometimes "reverted color" to a pale cartridge-buff; but, at maturity, changed to a pale pinkish-orange due to the separation of numerous conidia broken from the sporogenous heads. These conidia budded copiously, giving the appearance of a bacterial contamination.

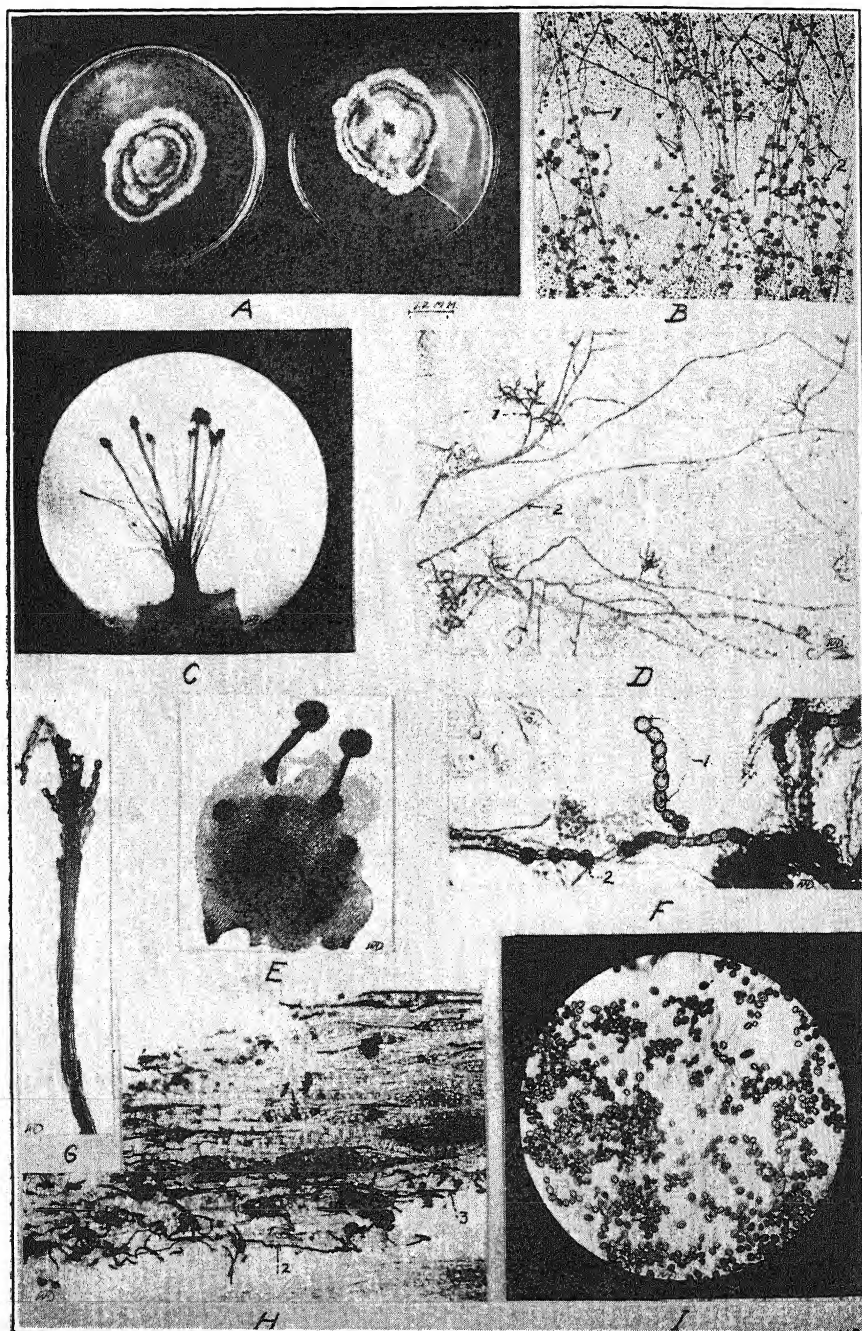


PLATE I. Photographs of cultures, fruiting bodies, spores and hyphae of *Sporocybe azaleae*.

A. Two immature, monosporous cultures transferred to potato-dextrose agar on February 14; incubated at 22° C. and photographed the following March 10. Dark concentric areas locate the penicilloid form of sporulation.

Transfers from pink cultures, incubated at 28° C., produced a moist, strict growth with pionnotid and penicilloid forms of *Sporocybe*.

Four nonsporulating "albino" strains of this fungus were isolated but were not employed in the experimentation. They, however, were transferred and retained in culture for 2 years without sporulation. They are considered as a spermogonial form.

Sclerotia were formed on the surface by hyphae emerging from substrata such as agar, azalea buds, stems, leaf petioles, and floral parts (Fig. 1, M and Plate I, E). In the presence of sufficient sunlight and air, they generally formed on the surface of the receptacle, uncovered and covered by the agar, but they may form also on other materials adjacent to nutrients and in host tissues. Small sclerotia formed in one culture prepared by spreading coremiospores on cherry-dextrose agar and incubating in van Tieghem cells. After 48 hours, the germ tubes had branched and intertwined, forming sclerotia (stromata) averaging 35 μ in diameter and 1.8 to 10 μ thick. They first appeared in cultures as gray flecks with minute white centers, but later became black and papillate, and measured 18 to 65 μ in diameter. Some of the papillate sclerotia formed matured coremia. Fully matured sclerotia in and on host tissues differed in size, shape, and structure. On old bud scales, they averaged 84 μ in height and 109 μ in width.

A large typical sclerotium was composed of layers: epidermal and cortical, and a central core containing most of the viable cells distinguishable by vital staining. The core was generally the source of the coremial stipe (Fig. 1, O).

After overwintered sclerotia had been incubated for 24 hours in a suitable environment, sturdy hyphae emerged through their apices and formed mycelial colonies about 0.5 mm. in diameter. Tests showed that sclerotia remained viable on azalea buds for 2 years and generally produced but 1 coremium annually; however, two coremia have been observed on a single

B. False spore "heads" of the cephalosporic stage, cultured on potato-dextrose agar; 16–20 μ in diameter; strain D; set July 4 and photographed July 28. 1. Individual spores showing in a "head." 2. "Slime" conceals individual spores, which formed in most of the "heads."

C. A partially dissected coremium showing a synema (stalk) composed of parallel hyphae; a caput (head) of coremiospores; a sclerotium (subiculum) supporting the stipe and the attached bud scale; see also G.

D. Conidiophores of the penicilloid stage bearing chain conidia; a stage following the formation of cephalosporae; a culture prepared July 27 and photographed on August 9. A region located in one of the dark concentric rings shown in A. 1. Conidiophore of the penicilloid type bearing conidia in chains. 2. Cells in a vegetative hypha.

E. Coremia and sclerotia in 1933 bud scale tissues of *Azalea nudiflora*; photographed September 6, 1934. Mounted in lactophenol-green.

F. Chlamydospores cultured on potato-dextrose agar in a van Tieghem cell; incubated from April 15 to June 17; average diameter 7.2 μ . 1. Vertical spore chain. The positive was partially bleached and traced with ink. 2. Chlamydospores as photographed.

G. Portion of a synema (stalk) of a coremium showing its parallel conidiophores (hyphae) and terminal conidia in chains.

H. Vegetative hyphae in an azalea stem; tangential, free-hand section just below an infected bud; stained and mounted in lactophenol-green. 1. Tyloses in cells of the medullary ray. 2–3. Hyphae are both inter- and intracellular.

I. Photomicrograph of coremiospores mounted in lactophenol-green July 29; average diameter of spores 5.4 μ .

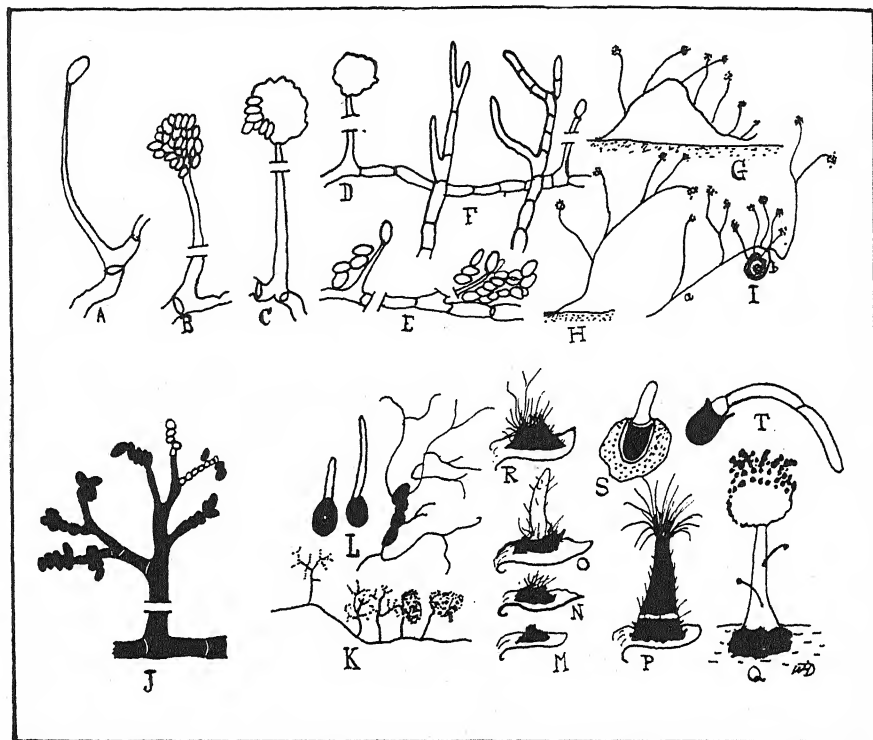


FIG. 1. Tracings of camera-lucida drawings showing the cephalosporic, penicilloid, sclerotial, and coremial stages of *Sporocybe azaleae*. A. A conidiophore with its terminal cell abstricted thereby forming the first conidium of a "false head" or caput. B. Abstricted conidia forming a caput. C. A matured caput with conidia in a "drop-let" or false head; conidia $2-4 \times 3-5 \mu$. D. Arrangement of conidiophores on a sporulating hypha cultured on cherry agar; conidiophores 63 and 44μ long. E. Conidia in two "false heads" separating after drying for 4 months. F. Two hyphae showing the sizes of cells and method of branching after they had been incubated for 56 hours on cherry agar in a van Tieghem cell. At the left, a young actively growing hyaline hypha near the margin of the culture; branches projected at an angle of 30° . At the right, a matured hypha; cells with dark walls and shorter in length; 40 , 72 , 10 , 55 , 50 , 50 , and 50μ , respectively; cells in A averaged 3 times this length. G, H, I. Arrangement of conidiophores on sporulating hyphae. Few conidiophores branched as I, a; basal portion 16μ bearing branches or forks 32μ long. Whorls are often formed and here conidiophores are more numerous as I, b. Air currents raised part of 8 above the agar. J. A dendritic or penicilloid conidiophore formed in culture after incubated for 30 days on potato-dextrose agar; 66μ tall; conidiophores and conidia were a smoky color when matured but nearly hyaline when first formed and averaging 5μ in diameter. K. Arrangement of dendritic conidiophores on a sporulating hypha. Distances between the first two conidiophores at the left, 100μ ; others averaged 67μ apart. L. Conidia of the penicilloid form germinating after incubating for 12 to 48 hours. M-Q. Stages in the formation of a coremium from a sclerotium, during April. M. Overwintering sclerotium on a bud scale. N-O. Emergence of coremial hyphae. P. The caput, forming from the hyaline hyphal tips, while the basal portions are dark colored. Q. A matured coremium with the sclerotium; synema, stalk or stipe; caput, with coremiospores borne both singly and in chains, but mostly in chains. Partially diagrammatical. R. Renewed growth in an old sclerotium which had borne one coremium. S-T. Coremiospores germinating in water after incubating for 12 hours. S. Germ tube emerging from a coremiospore; exosporium surrounded by a slime capsule. T. Coremiospore incubated for 24 hours; slime capsule dissolved.

sclerotium. Thus, the sclerotia are reproductive bodies, which tide the fungus through adverse conditions and form either coremia or mycelium. Although these structures are considered stromata by some, the writer prefers to designate them as sclerotia (Fig. 1, M-P).

THE CEPHALOSPORIC STAGE

Cephalospores were observed on sclerotia, coremia, between bud scales, on infected azalea buds, rotting bark, old infected stems, decomposed floral parts, nutrient solutions and media. Cephalospores formed in "false heads" when coremiospores were incubated on water agar for various periods. In some of these cultures, hyphae from germinating coremiospores came in contact with each other, intertwined, and formed a spiral from which phialides (conidiophores) grew singly and vertically. These phialides were neither delimited by cross walls within themselves nor by partitions at their junction with the "parent hyphae"; old ones, however, sometimes bore cross walls. They were often larger in diameter at the base than at the apex and of varying lengths (Fig. 1, A-I, and Plate I, B).

PENICILLOID STAGE

Penicilloid conidiophores formed on the nutrient agar (Fig. 1, J-K, and Plate I, D) of each cephalosporic culture that had been incubated for one to three weeks or had been exposed to desiccation. This stage was observed also in each monocoremiospore culture on diseased floral parts, buds, twigs, and fruits. Its incidence in hundreds of monocoremiospore cultures gave evidence that it was a conidial form following the cephalosporic stage. Germinated conidia produced forms identical with those produced by coremiospores.

The conidiophores were formed singly, were dark-brown, and grew vertically from a parent, sporulating, prostrate hypha. They bore an unbranched base or trunk with a spreading, dendritic caput composed of sporogenous branches with the spores attached end to end. Drying conidia changed from a light-brown to sepia, abstricted readily and germinated in tap water.

It is to be noted that each conidiophore with its branches appears to be an independent sporiferous element of a coremium. It originates from a sporulating hypha rather than from a sclerotium and develops singly rather than in a group or synema. These conidia remained intact for a longer period than those in a compact head. Since the conidiophore with its conidia has a slight resemblance to *Penicillium*, it may be known as a penicilloid stage or form of *Sporocybe azaleae*.

THE COREMIAL STAGE

Most of the coremia formed on the scales of flower buds but they were also observed on leaf buds, twigs, leaf scars, petioles, flower pedicels, floral parts, capsules, and excised host parts lying on soil. Coremia also de-

veloped on most of the media employed for culturing the fungus, but sterilized, wet filter paper in Petri dishes was the substratum most commonly employed. By using inoculum from a monosporous strain, C-13, coremia formed after incubating the agar cultures at room temperatures for 2 to 6 months. Potato dextrose agar, steamed azalea buds, twigs and leaf petioles were favorable substrata but malt and prunes were unsuitable on account of their rich sugar content.

Most of the coremia developed from the centers of small sclerotial bodies or subicula (Fig. 1, M-Q, and Plate I, C and E). In bud scales, they averaged 109μ wide and 84μ in thickness. From the center of each sclerotium, a stipe or synema appeared first as a conical papilla of light-gray hyphae emerging at right angles to the surface of the sclerotium (Fig. 1, O). Hyphae at the center of the stipe grew more rapidly than those at the periphery where sporulating hyphae became free. Finally, a head or caput was formed at the apex of the parallel hyphae of which the stipe is composed. The stipe changed from a light-gray to brown while the immature caput remained gray but when both were fully matured, they were a dark-brown or appeared black to the unaided eye. The tips of these hyphae consisted of conidia or conidiophores, mostly borne in chains but sometimes single. Dissection showed these conidiophores may branch similarly to the penicilloid form. The weight and flexibility of the conidal chains caused hyphal tips in the synema to droop so as to form the caput (Fig. 1, P).

The cultures of *Sporocybe azaleae* were especially interesting, since the fungus grew slowly over the surface of the agar or about 4 mm. daily, changed from a light-gray to a dark-olive color and bore rings of sporulation induced by alternate periods of light and darkness. They produced several forms of fruiting bodies that bore spores of the penicilloid form, the hyphae penetrated the agar and, by reflected light, the mycelium was dark purple.

THE ASCOGENOUS STAGE

Coremia on infected azalea buds were collected from New Hampshire, New York, Massachusetts, Connecticut, New Jersey, and Virginia. From each of these collections, monosporous cultures were incubated on azalea buds, stems, agars, and many other substrata but did not form perithecia. However, when azalea stems were properly inoculated with two or more strains determined by trial, perithecia formed. From 50 trials, 4 strains were isolated, which, when crossed, produced immature perithecia. Perithecia also were observed on old infected buds that had been incubated 6 months in Coplin breeding jars.

These strains were mated in Petri-dish cultures and stored in damp chambers, but subsequently destroyed by forces uncontrolled by the investigator. The few ascospores, however, that had been obtained by preliminary mating tests were employed in experimentation. The ascospores failed to germinate immediately, but seemed to need an after-ripening period of

several months. Tests of 4 cultures produced a few germinating ascospores that were implanted on potato-dextrose agar. Each of these cultures produced typical cephalospores and penicilloid stages of this fungus. Further investigation, however, is needed to obtain sufficient data.

Measurements in microns of 8 perithecia averaged as follows: Beaks, width 67; length 902.4. Body height 394.8; width 310.8. Spores, $1.8-3.6 \times 3.6-9$. One perithecium with a broken beak bore a few mature spores; all other perithecia were far less mature. It is to be noted that the length of the beaks was more than twice the height of the body. This morphological character is similar to that of *Ceratostomella ulmi*, which, as previously stated, is closely allied to *Sporocybe azaleae*.

Another attempt is being made to determine strains and produce the ascogenous stage, which, in turn, will produce the conidial forms. Furthermore, the question of ascospore germination must also be solved.

DIAGNOSIS

Cephalosporic form of *Sporocybe azaleae*: The cephalospores formed on upright conidiophores, or phialides.

Phialides: Arising singly and vertically from young, horizontal, prostrate hyphae; hyaline to gray; seldom branched but formed singly; lengths, 12 to 30 μ , average diameter at the base 2.5; at the apex 1.7 μ . Function: formation of conidia.

Cephalospores: Formed singly as conidia at the apex of phialides only, ellipsoidal and hyaline when forming but oblong-angular and a tint of brown or gray when mature and dry. Sizes: limits of variation, $2.5-4.2 \times 5.1-9 \mu$. Standard, $3.5 \times 5.5 \mu$ (100 fresh spores measured in culture). Function: dissemination and reproduction.

Caput: A "false head" at the apex of the phialide, averaging 20 μ in diameter and formed by the conidia cohering when abstricted but separating ultimately. Assigned to *Cephalosporium* sp.

Penicilloid form of *Sporocybe azaleae*: Observed in cultures following cephalospores and on decaying infected azalea buds, flowers, and branches.

Conidiophores: Arising vertically from a horizontal sporulating hypha, average spacing 76 μ apart, dendritic, base or unbranched trunk, 8 to 15 cells, averaging 60 μ in height, and 3-6 μ in diameter.

Caput: Composed of 2 to 12 spreading, dichotomous branches or conidiophores each supporting a chain of conidia at the tip.

Conidia: Unicellular, catenulate or formed in chains of 6 to 10 individuals composing 45 μ of the tips on each branch of the dendritic conidiophore. Dried conidia are unicellular, ovate to globose; often slightly apiculate, brown; sizes $1.5-5 \times 2.8-9 \mu$. When matured on agar, 70 per cent were nearly globose and averaged 5 μ in diameter. When germinated in water on nutrient agar, they form typical *Sporocybe* cultures.

Coremial Stage of Sporocybe azaleae: Coremia found mostly on the terminal flower buds of azaleas, infected the previous season, or that have remained attached to the host one or more years.

Synema or Stipe: It originates from a sclerotium averaging $109 \times 84 \mu$ when in the bud scales. At first, gray, but sepia when mature; 50.4 μ in diameter and 480 μ in height (average of 10 matured synemae); seldom, if ever, branched; consisting of cohering parallel hyphae or conidiophores.

Caput: Composed of the sporulating free ends of hyphae from the synema; hyphae fail to cohere on account of the spores but bend at their apices; height 106 μ ; expanded width 188 μ (average of 10 specimens). At first, gray, then sepia.

Coremiospores: Formed in chains, 1 to 9 at the tips of conidiophores in the caput; when dried, the chains are broken so as to form individual spores; seldom, 2-celled; globose-ovate, ellipsoidal and oblong; color varies from gray, when formed, to dark-brown or sepia when mature; sizes, limits of variation $3-10 \times 3-17 \mu$; standard, 5.4×7.2 ; deviation $0.638 +$ (100 fresh spores collected from coremia on azalea buds in the open, May 1935). These spores decreased about 20 per cent in size upon drying; may increase 300 per cent in diameter while incubating during germination; viability, one year.

SUMMARY

Mycologists have described previously only the coremial stage of *Sporocybe azaleae*, but have placed this fungus in keys adjacent to *Graphium ulmi*, which has cephalosporic, coremial, and ascogenous stages. This investigation was undertaken to determine whether *Sporocybe azaleae* likewise possesses these stages in its life cycle.

Measurements of the hyphae and sclerotia, together with descriptions, are recorded and cultures of the fungus briefly described.

The fungus possesses the following stages: mycelial, chlamydosporic, cephalosporic, penicilloid, coremial, and probably ascogenous. Albino strains and pionnotes also were observed in cultures.

Cephalospores formed in cultures obtained by incubating coremiospores on various substrata.

A dendritic or penicilloid form of sporophore developed on nutrient agar cultures directly following the cephalospores. Conidia that formed on the tips of these dendritic sporophores were similar to those formed on coremia.

Coremia developed from sclerotia that had formed in the flower-bud scales of azaleas. After overwintering, the sclerotia germinated, forming coremia. Morphologically, a coremium comprises an aggregate of penicilloid conidiophores bearing conidia similar in genetic content, size, shape, and color to those of the penicilloid form.

Perithecia were obtained by mating certain strains found among isolates from various localities. The ascospores appeared to require an after-ripening period, the length of which was not determined because the cultures were wantonly destroyed. The perithecia obtained in culture bore long beaks similar to those of *Ceratostomella ulmi* and measurements are recorded in the text. The evidence thus far obtained was insufficient to establish beyond doubt that this is the perfect stage of *S. azaleae*, so the work is being repeated.

From the above, it is to be noted that *S. azaleae* has cephalosporic, coremial, and ascogenous stages similar to those of *Ceratostomella ulmi*. However, its cephalospores, coremiospores, chlamydospores and ascospores were dark colored when mature. In addition, *S. azaleae* possesses sclerotia, chlamydospores, and a penicilloid form.

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VARIATION IN PATHOGENICITY AND CULTURAL CHARACTERISTICS OF THE COTTON-WILT ORGANISM, *FUSARIUM VASINFECTUM*¹

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INTRODUCTION

The investigations herein described concern variations of *Fusarium vasinfectum* Atk. as to cultural characteristics and pathogenicity to cotton. A possible explanation also is given of the phenomenon that a variety of cotton may show greater resistance to the *Fusarium* wilt organism in some localities than in others.

Isolations of the fungus have been made over a period of years from hundreds of diseased plants collected at various localities in South Carolina. A limited number of these isolates were used for experimentation. Selections were made on the basis of differences in cultural characteristics, as well as length of time the isolates had been retained in culture.

VARIATION IN CULTURAL CHARACTERISTICS

Materials and Methods

Thirteen monosporial cultures of *Fusarium vasinfectum* were used. Three of them were derived from fresh isolations from cotton plants that had wilted in the field in 1937. The remainder were cultures originally isolated in 1931 to 1936, but recovered in 1937 from cotton plants infected in the pathogenicity experiments (described later in this paper). Eight to ten monoconidial cultures had been made from each of these isolates. These monosporial cultures showed the same characteristics as the respective mother cultures and only one of each was selected.

The technique employed was essentially that used by Ullstrup³. All cultures were grown on nonacidified potato-dextrose agar and kept at 28° C. Subcultures were made by successive transfers of masses of inoculum from one agar slant to the next, at first weekly, and later bi-weekly until 17 such transfers were made. At each transfer, spore suspensions were prepared and 3 to 6 single spores from each culture were transferred to Petri dishes with potato-dextrose agar. By observing these single-spore isolates, variants could be distinguished as they appeared.

Results

Figure 1, A, shows the appearance of 13 isolates at the beginning of the experiment; figure 1, B, the appearance of these isolates after 17 transfers,

¹ Technical Contribution No. 71 from the South Carolina Agricultural Experiment Station.

² Agent, Division of Cotton and Other Fiber Crops and Diseases; formerly Assistant Pathologist, South Carolina Agricultural Experiment Station.

³ Ullstrup, A. J. Studies on the variability of pathogenicity and cultural characters of *Gibberella saubinetii*. Jour. Agr. Res. [U. S.] 51: 145-162. 1935.

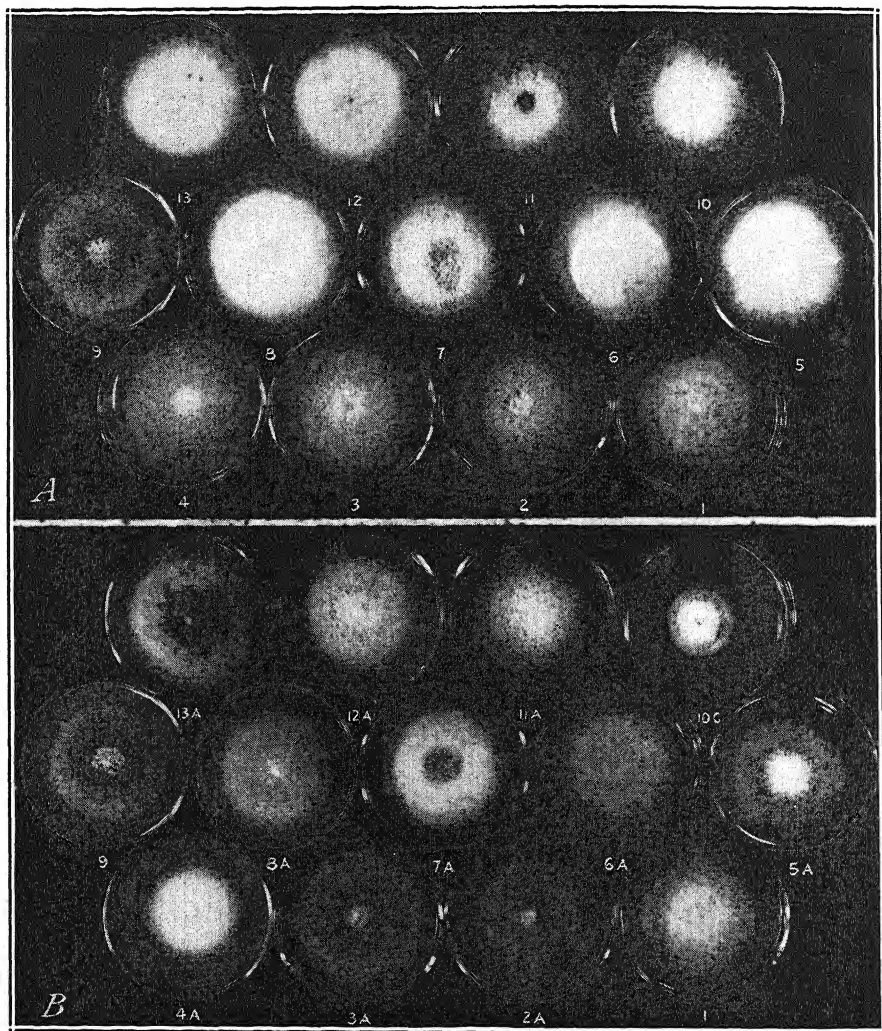


FIG. 1. Variation in the cultural characteristics of *Fusarium vasinfectum*. A. Thirteen different isolates. B. Variants that arose within 17 transfers from isolates represented in A. (Cultures are arranged in the same order in A and B.)

arranged in the same order as in figure 1, A. Eleven of the 13 isolates showed variation in cultural characteristics.

Variations were chiefly in 2 directions; namely, decrease in abundance of aerial mycelium and decrease in the rate of radial growth. No changes occurred in the opposite direction. However, because of the decreased rate of radial growth, some variants appeared to have had denser aerial mycelium than the parent culture.

The occurrence of variation among the respective isolates was irregular, some isolates giving rise to variants at the second transfer but others remaining constant until the 16th or 17th transfer.

Variants generally remained constant in cultural characteristics, but some of them developed secondary variants. Variants had a tendency to dominate the original, even to apparent exclusion of the latter.

An additional experiment was run in which continuous monosporous transfers were made from a single original isolate. The variants appearing in this experiment differed from the original mainly in the rate of growth.

VARIATION IN PATHOGENICITY

Materials and Methods

In 1937, ten monosporial isolates of *Fusarium vasinfectum* were used to infest soil in which the cotton varieties Farm Relief 2, Semi wilt, and Super Seven were grown. In 1938, 1 recent isolate and 4 cultural variants were added and all were used with the varieties Farm Relief 2 and Dixie Triumph 12. Soil, from a field where cotton had not been grown for at least 5 years, was heavily infested with cultures of the pathogen grown on an oat-wheat mixture.⁴ Five 2-gal. pots were used for each isolate and cotton-variety combination. Twenty-five seeds were planted in each pot. A month later, the young plants were thinned to 5 per pot. As soon as a plant exhibited pronounced wilt symptoms, it was removed, examined for internal symptoms, and then a portion of the stem, from just above the ground line, plated on acid potato-dextrose agar. At the conclusion of the experiment, all remaining plants were removed and treated similarly.

Results

Data were obtained from 20 to 40 plants per isolate, the average number being 25. The following considerations are concerned primarily with the expression of external symptoms on the susceptible Farm Relief 2 (Fig. 2).

Isolates that had been long kept in culture were less pathogenic than those of recent origin. Isolates 1 and 2 were obtained in 1931, 3 and 10 in 1932, and the remainder in 1936 and 1937.

Four cultural variants were added in 1938. (These are indicated by letters following the number given to the parent isolate.) Variants 5 Z and 5 A were markedly less pathogenic than the parent. On the other hand, variant 12 A and 8 A deviated to a lesser extent from their respective parents.

The relative degree of pathogenicity of the various isolates remained of the same general order in the two successive years. The fungus was re-isolated from diseased plants in the experiments in 1937 and monosporial isolates were made. These isolates were used in the experiments of 1938, excepting No. 12, which was a new isolate.

The single passage of the pathogen through the host plant in 1937 did not significantly modify its relative pathogenicity as expressed in 1938. No

⁴ Oat-wheat mixture; 1 bushel of oats and $\frac{1}{2}$ bushel of wheat were ground together. To this was added $\frac{1}{2}$ bushel of unground oats and the whole moistened to a crumbly consistency. Transfers of the respective isolates were made to one-quart flasks half-filled with the mixture. The cultures were allowed to grow for 10 days. Inoculum and soil were mixed in the proportions of 1 to 40 by volume.

correlation could be made between the locality from which an isolate originated and its degree of pathogenicity. Isolates 6 and 7 originated from Clemson, in the upper northwest portion of South Carolina; the remainder originated from the lower coastal-plain region of the State.

Positive identity of all slight internal symptoms left correlations between pathogenicity and the presence of internal symptoms open to some question. The same held true on the basis of reisolation of the fungus. One could not be certain in all instances that failure to recover the fungus by plating a portion of the stem could be attributed to complete absence

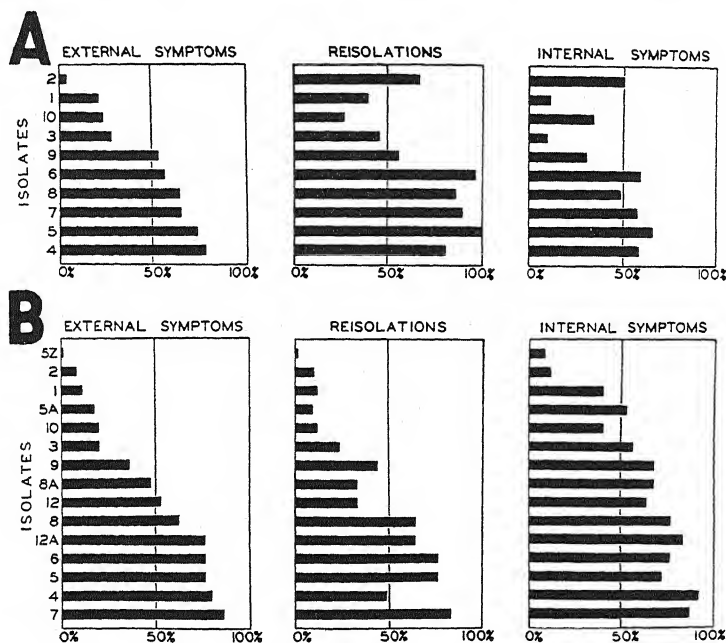


FIG. 2. Relative pathogenicity of different isolates of *Fusarium vasinfectum* to the cotton variety Farm Relief No. 2, expressed on a percentage basis. A. Results obtained in experiments of 1937. B. Results from parallel experiments in 1938 with one recent isolate (12) and three cultural variants (numbers followed by letters).

of the fungus in the plant. Nevertheless, the results obtained are expressed in figure 2. In general, the fungus was recovered, in 1937, from a higher percentage of plants than showed either internal or external symptoms. The experiments of 1938 were started a month earlier than those of 1937, and symptoms of the disease were not expressed until the plants were somewhat older. The lower percentage of recovery of the fungus in 1938 may have been due to greater difficulties in reisolation. The fungus was slower in emerging from the older wood and contaminations were more abundant.

The cotton varieties Dixie Triumph 12, Super Seven, and Semi wilt proved to be much more resistant to the *Fusarium* isolates than did Farm Relief 2. This accords with field observations in general. Based on the

percentage of plants exhibiting external symptoms and from which the pathogen was recovered, the degrees of susceptibility of Dixie Triumph 12, Super Seven, Semiwilt, and Farm Relief 2 were found to be in the order of 2, 3, 4, and 10, respectively. In figure 2 the isolates are arranged in the order of pathogenicity to Farm Relief 2. In general, their pathogenicity to the other 3 varieties followed somewhat the same order, though the percentages of infection and external symptoms in the resistant varieties were so reduced that differences between isolates were less evident.

The number of reisolutions made in 1937 from Super Seven and Semiwilt were far in excess of the number of plants exhibiting either external or internal symptoms. For example, isolate 6 was recovered from 100 per cent of the Super Seven plants, but only 28 per cent of the plants exhibited external symptoms and 32 per cent internal symptoms.

A spore suspension was used as inoculum in a parallel experiment in 1938, mixing about 5 billion spores into the soil of each pot. The spores were washed from cultures grown on the oat-wheat mixture. Five isolates (Nos. 5, 6, 8, 9, and 5 Z) of the pathogen were used with the variety Farm Relief 2. In general, the relative pathogenicity of the isolates followed the same order as in the experiments previously described. However, the percentages of infected plants, as well as of plants exhibiting external symptoms, were considerably lower.

DISCUSSION

Based on the expression of external symptoms by the cotton variety Farm Relief 2, the isolates of *Fusarium vasinfectum* presented a wide range in pathogenicity. It has been reported for certain other *Fusaria*^{5,6} that isolates characterized by a combination of abundant aerial mycelium and rapid radial growth are highly pathogenic. This held true for the isolates 5, 6, 8, and 12. On the other hand, isolates 4 and 7 were relatively high in pathogenicity but exhibited an appressed type of growth on potato-dextrose agar. Isolates 2, 1, 10, and 3, with the appressed cultural character, were lowest in pathogenicity. These phenomena are in accordance with studies of other *Fusaria* and lead to the conclusion that a cultural variant may or may not be less pathogenic than the isolate from which it arose.

It was observed that fresh isolations of *Fusarium vasinfectum*, obtained from diseased plants in the field, are generally characterized by abundant aerial mycelium and rapid growth in culture. In all probability, the very weakly pathogenic isolates 2, 1, 10, and 3 with the appressed cultural character represent variants that had arisen during their lengthy period in culture.

Pathogenicity and cultural characteristics of the isolates were not affected to any extent by a single passage through the host. This is especially

⁵ See footnote 3.

⁶ Harvey, C. C. Studies in the genus *Fusarium*. VII. On the different degrees of parasitic activity shown by various strains of *Fusarium fructigenum*. Ann. Bot. 43: 245-259. 1929.

interesting with respect to the weakly pathogenic isolates that had long been in culture.

The cotton varieties Dixie Triumph 12, Semi wilt, and Super Seven proved to be considerably more resistant to all the isolates than did Farm Relief 2. Reisolation of the fungus from the resistant varieties was far in excess of the number of plants exhibiting external symptoms. This would indicate that resistance was not involved in the infection process.

Whether or not the development of cultural variants differing in pathogenicity answers the problem of why a variety of cotton seems to be more resistant in some localities than in others may be open to question. Edaphic factors may be of significance. Nevertheless, since this fungus grows saprophytically in the soil, it is possible that variants differing in pathogenicity may occur in the field. Orton⁷ obtained "dissociation" of *Fusarium vasinfectum* in sterilized soil.

SUMMARY

Evidence is given that variants of *Fusarium vasinfectum* do arise in culture. A variant tends to have less aerial mycelium and to exhibit a slower rate of growth than the parent. None was found that reverted to the parent type during 17 successive transfers. Moreover, a variant appeared to dominate the parent type, even to the exclusion of the latter. A variant may give rise to secondary variants.

Isolates showed a wide range in pathogenicity. Cultures that exhibited abundant aerial mycelium and grew rapidly were among the highly pathogenic group, but variation from this cultural type may or may not be paralleled by decrease in pathogenicity.

Isolates that had long been retained in culture were weakly pathogenic. Their cultural characteristics indicated that they were variants that had arisen in culture.

A single passage of an isolate through the host did not modify its pathogenicity.

The high percentage of reisolation from resistant cotton varieties suggests that the mechanism of resistance is not involved in the process of infection.

It is suggested that variants of this fungus, which differ in pathogenicity, may occur in the field.

SOUTH CAROLINA EXPERIMENT STATION,
CLEMSON, SOUTH CAROLINA.

⁷ Orton, C. R. The dissociation of *Fusarium* in soil. Bull. Torr. Bot. Club 62: 413-418. 1935.

THE PRACTICABILITY OF DETECTING DUTCH ELM DISEASE BY TRUNK SAMPLING

W. E. AHRENS

(Accepted for publication January 30, 1940)

The purpose of this study was to devise a practicable method for diagnosing Dutch elm disease by sampling accessible portions of the trunks of suspected elms, and to determine some applications of this method in the control of the disease.

There are limitations to the technique of finding all elms infected with *Ceratostomella ulmi* (Schwarz) Buisman, by culturing twig samples from trees expressing foliar symptoms commonly associated with the Dutch elm disease. True and Slowata¹ found that from 0.007 to 2.3 per cent of such elms in plots sampled in 1936 and 1937 were actually infected with *C. ulmi* and, conversely, that 50 per cent of the trees found infected did not show external symptoms. In addition, collecting twig samples from elms is expensive, since approximately one-half of them are so tall that satisfactory samples cannot be obtained without climbing the trees. Also an accurate inspection for external symptoms is impracticable in densely wooded areas. Furthermore, any scouting method based on the presence of foliar symptoms is handicapped by the shortness of the period during which it can be employed.

The occurrence of both the fungus and the vascular discoloration associated with it in the trunks of infected trees has been mentioned by earlier writers^{2, 3} and extensively demonstrated by Smith.⁴ Therefore, the principal problem in the present study was to determine whether specimens suitable for laboratory diagnosis could be collected from the trunks of elms suspected of having the disease and whether such a procedure would be a practicable method for finding infected elms, particularly during the dormant season. In the course of the study suitable tools were devised, methods for trunk sampling were developed, the effectiveness of the method for finding diseased trees was tested in the field, and the effects of the trunk-sampling operation upon the trees were determined.

MATERIALS AND METHODS

An arch-type leather punch, extracting a sample $\frac{3}{4}$ in. in diameter, was used throughout the early part of the work. Later, an inexpensive leather punch having a bore $\frac{1}{2}$ in. in diameter was found satisfactory. The

¹ True, R. P., and S. S. Slowata. Scouting and sampling elms with symptoms commonly associated with the Dutch elm disease as an aid in eradicating *Ceratostomella ulmi*. *Phytopath.* 29: 529-537. 1939.

² May, C., O. N. Liming, and Thelma Alexander. The Dutch elm disease in Ohio. (Abstract) *Phytopath.* 21: 125. 1931.

³ Schwarz, Marie B. Das Zweigsterben der Ulmen, Trauweiden, und Pflsichbäume. Utrecht. A. Oosthoek, pp. 7-32. 1922. Part relating to elm translated by L. K. Kelsey as "The twig wilt and vascular disease of the elm." Bartlett Research Lab. Bull. 1: 5-25. 1928.

⁴ Unpublished report. A. L. Smith, formerly Field Assistant, Division of Forest Pathology, Bureau of Plant Industry.

punch was driven into the tree with a 1-pound composition rubber mallet, given a downward thrust to snap off the wood core inside it, and then removed from the tree (Fig. 1, A). The wood core was ejected from the punch with a wooden plunger set in the handle of the mallet.

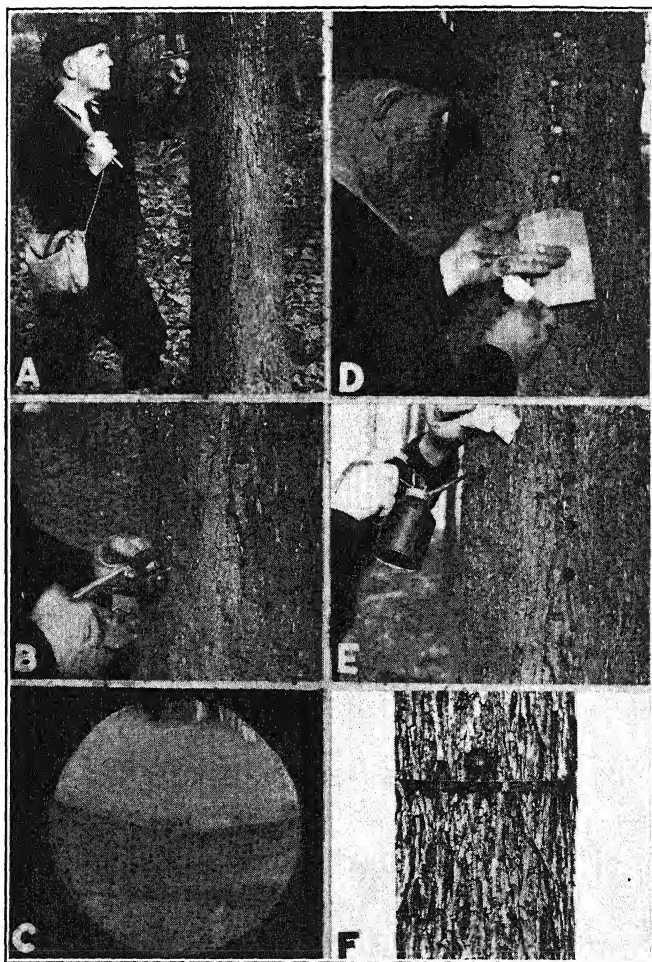


FIG. 1. Steps in trunk sampling and a healed injury. A. Removing samples. B. Slicing sample previous to inspection for vascular discoloration. C. Typical vascular discoloration in sliced trunk sample. D. Collecting samples for culturing. E. Painting the wounds. F. Healed wound 1 growing season after sampling.

The samples were taken on the circumference of the tree at a height convenient to the operator, usually the shoulder line. The spacing of the sampling points was based on unpublished studies by Smith⁵ of the distribution of vascular discoloration in trunks of infected elms. He examined cross sections of 284 elms showing Dutch elm disease and found that 242 (85 per cent) contained some discoloration at breast height. By examining

⁵ See footnote 4.

incisions spaced at 3-, 6- and 12-in. intervals on the circumference of these 242 trees, he detected vascular discoloration at one or more points in 95.5, 93, and 78 per cent of the trees, respectively. Since incisions at 3-in. intervals were only 2.5 per cent more effective than those at 6-in. intervals, samples taken by the author during the dormant season were spaced approximately 6 inches apart.

Because of variation in growth increment and thickness of bark, the sampling depth was based on the number of annual rings penetrated rather than on a linear measure. The internal symptoms of the disease often occur in the wood of annual rings infected prior to the current year; therefore, the samples included wood from no less than 2, but usually no more than 5, annual rings.

All the samples were sliced diagonally through the center and across the grain (Fig. 1, C). Those $\frac{3}{4}$ in. in diameter were sliced with a pocket knife; those $\frac{1}{2}$ in. in diameter were sliced with especially adapted pruning snips (Fig. 1, B).

When discoloration was detected in a sliced sample, the subsequent discolored samples from the trees were retained without slicing. In most cases only 1 or 2 plugs from each tree contained discoloration and, therefore, 3 additional samples were taken in a vertical line with the hole from which the sliced discolored plug was removed (Fig. 1, D). After the outer bark had been removed from these samples, they were placed in a glassine bag, properly labeled, and later cultured on potato-sucrose agar. The punch was sterilized with alcohol if discoloration was found.

A pump-type oiling can was converted into a suitable instrument for applying wound dressing to the $\frac{1}{2}$ -in. in diameter holes from which samples had been taken. The position of the exit holes on the nipple and the pumping action spread the paint evenly over the inside surface of the wound (Fig. 1, E).

EFFECTIVENESS OF TRUNK SAMPLING

A. Sampling During the Dormant Season

During the dormant season of 1936-1937, samples were taken at 6-in. intervals from the trunks of 6,031 elms to study the effectiveness of this method for finding infections of *Ceratostomella ulmi*. The elms sampled had been inspected for foliar symptoms during the previous growing season, and 114 of them being found diseased, were eradicated before the trunk sampling was done. Elms smaller than approximately 3 in. in diameter, breast high, were not trunk-sampled because twig samples could be collected in less time. The results of this study are given in table 1.

Forty-five new *Ceratostomella ulmi* infections were discovered within the plots. These increased the total number of cases for the period of the study from 114 to 159. Extensive patterns of vascular discoloration in the 45 trees indicated that they had been diseased during at least the final scouting period preceding trunk sampling. There were occasional

TABLE 1.—Additional infections of *Ceratostomella ulmi* found by trunk sampling dormant elms at 6-inch intervals in areas from which recognized infected elms had been removed during the previous summer

Plot name and location	Date of sampling trunks	Trees trunk-sampled			Infected trees eradicated during previous summers	Total infected trees found	
		Number	Number cultured	Number yielding <i>C. ulmi</i>		Number	Percentage found by trunk sampling
Convent, Morris Co., N. J.	October 1936	1,036	123	23	49	72	32.0
Morris Co., N. J. (Assorted plots)	January–March 1937	1,183	106	1	0	1	100.0
East Orange Water Reservation, Essex Co., N. J.	January 1937	294	16	0	1	1	0
Milltown, Somerset Co., N. J.	January 1937	251	31	13	47	60	21.7
Raritan, Somerset Co., N. J.	January 1937	569	50	3	5	8	37.5
Westchester Ave., Westchester Co., N. Y.	January 1937	309	24	0	2	2	0
Armonk, Westchester Co., N. Y.	January 1937	350	16	0	0	0
Congers, Rockland Co., N. Y.	March 1937	1,373	59	4	6	10	40.0
Nanuet, Rockland Co., N. Y.	March 1937	666	14	1	4	5	20.0
Total		6,031	439	45	114	159	28.3

a From records of Dutch Elm Disease Eradication Office, Bureau of Entomology and Plant Quarantine.

galleries of *Scolytus multistriatus* Marsh. and *Hylurgopinus rufipes* Eich. in many trees. Adults of the former had recently emerged from heavily diseased portions of 3 trees.⁶ Probably those 3 and possibly others among the 45 had been instrumental in further spread of the fungus.

That trunk sampling is not a method for finding all *Ceratostomella ulmi* infections was demonstrated by discovery of 4 diseased trees among 80 elms removed from the Milltown plot after the trunk sampling was completed. However, vascular discoloration in those trees was not extensive.

The three foliar inspections of the elms in the Convent and Milltown plots during the summer preceding trunk sampling required 72 man days.⁷ Trunk sampling the plots consumed 28 man days.

These results suggest several ways in which trunk sampling during the dormant season could be employed in the control of Dutch elm disease: (1) to supplement summer scouting, particularly in areas in which an unusually large number of infected trees had been found by summer scouting; (2) to appraise the effectiveness of summer scouting by sampling plots selected at random; (3) to supplant summer scouting in wild or semi-wild areas adjacent to concentrations of valuable elms.

B. Sampling While Trees Are in Foliage

To determine the efficiency of trunk sampling as a method for collecting samples from trees expressing symptoms of Dutch elm disease, a study was conducted during the period June 16 to July 7, 1937, on 66 known diseased trees selected at random in Morris County, New Jersey. They were sampled at 3-in. intervals within 8 to 16 days after discovery by field scouts. *Ceratostomella ulmi* was isolated from trunk samples from 64 of the 66 trees sampled. Sampling at 6-in. intervals would have found 61, (92.4 per cent) of the trees.

Samples from 54 of the 66 trees (82 per cent) contained vascular discoloration in the 1936 annual ring. The circumferential distribution of this discoloration was such that approximately 75 per cent of the 66 trees could have been found by trunk sampling at 6-in. intervals during the preceding dormant period. However, in another group of trees only 31 per cent of 1,300 diseased trees removed later in the same season (July 7 to September 30, 1937) contained discoloration in the 1936 annual ring.

A further test of trunk sampling during the growing season was conducted during the period July 12 to August 25, 1937. Samples were taken in the usual manner at 6-inch intervals from approximately 5,500 elms not known to be diseased. They adjoined approximately 800 diseased trees discovered about 2 weeks earlier by foliar scouting and scattered throughout the known infected area in New Jersey. It is assumed that they were inspected for foliar symptoms at the time samples were taken

⁶ Trees were examined for insect infestation by W. D. Buchanan, Division of Forest Insects, Bureau of Entomology and Plant Quarantine.

⁷ Data supplied by Dutch Elm Disease Eradication Office, Bureau of Entomology and Plant Quarantine.

from the infected trees that they adjoined. Evidently they did not express external symptoms at that time, since no twig samples had been collected. *Ceratostomella ulmi* was isolated from trunk samples from 38 (0.7 per cent) of the trees.

Other studies yet to be published, indicate that as high as 20 per cent of the diseased trees found by summer scouting during 1937 did not contain sufficient trunk discoloration 1 to 2 weeks after symptoms were first observed to insure detection by trunk sampling at 6-inch intervals.

The results of the several studies of trunk sampling during the foliar season suggest two uses for this method in the control of Dutch elm disease: (1) To define the probable extent of vascular discoloration in more valuable infected elms to determine whether the infected parts could be excised; and (2) to speed summer scouting by collecting trunk samples from symptom trees that would otherwise require climbing.

Some Effects on the Elms

Measurements were taken of healing occurring after one growing season on wounds $\frac{3}{4}$ in. in diameter made by trunk sampling at 6-in. intervals. The 1,425 wounds were distributed on 469 trees in 19 plots, each containing 16 acres, in Morris County, New Jersey. Healing of all wounds, based on percentage of area calloused, averaged 71 per cent, 29 per cent were completely healed, and 75 per cent were closed 50 per cent or more.

Punches $\frac{1}{2}$ in. in diameter are recommended, but extensive data are not available on the healing of holes of this size. Alternate $\frac{1}{2}$ - and $\frac{3}{4}$ -in. samples, however, were taken May 14 to 17, 1937, from 10 trees at approximately 3-in. intervals on the circumference. Measurements of healing were made the following November. The percentages of the wounds made with the $\frac{1}{2}$ - and $\frac{3}{4}$ -in. punches that were completely healed were 52 and 21, respectively.

One year after the trees in the Morris County plots were trunk-sampled a study was made of the effect of sampling on growth increment. The average width (2.16 mm. \pm .11) of the 1937 annual ring formed on sampled trees the year following sampling was comparable to the width (2.23 mm. \pm .10) of the 1937 annual ring on unsampled check trees. Evidently the trunk-sampling operation had little effect on the increment of growth during the following year.

The relation between growth increment in 1936 and healing of wounds after sampling in 1937 was determined by comparing the width of the 1936 annual ring with the percentage healing of the wounds. On trees of average growth the wounds were approximately 84 per cent closed. The percentages of healing of wounds on trees below and above average radial growth were 50.7 and 94.0, respectively. One would, therefore, expect most wounds made with the smaller ($\frac{1}{2}$ -in.) punch on trees of average growth increment to heal in one growing season (Fig. 1, F).

Wood samples from trees previously trunk-sampled were plated to deter-

mine if fungi commonly associated with decay in living trees were present. None was found.³

SUMMARY

Satisfactory tools and methods have been developed for removing samples $\frac{1}{2}$ in. in diameter and suitable for culturing from trunks of elms suspected of having Dutch elm disease.

During the dormant season 1936-37 this sampling method was applied to 6,031 elms considered disease-free when last inspected for foliar symptoms in the preceding summer. The 45 new infections discovered constituted 28.3 per cent of all infections found in the plots by scouting for foliar symptoms and by trunk sampling.

Dormant-season trunk sampling was less expensive than intensive summer scouting.

Several studies of trunk sampling during the growing season indicated that approximately 80 to 92 per cent of infected elms showing foliar symptoms could be found by trunk sampling about two weeks after symptoms were observed. Those with vascular discoloration insufficient for detection were usually not severely affected by the disease.

Apparently trunk sampling during the dormant season at 6-inch intervals did not injure the trees, since (a) the growth increment of sampled trees during the year following sampling was not retarded; (b) none of the commonly recognized fungi associated with decay of living trees was isolated from platings of wood adjoining healed and unhealed trunk-sample wounds.

Five ways in which trunk sampling might be used in control of Dutch elm diseases have been enumerated.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

FUSARIUM LEAF SPOT OF SANSEVIERIA¹

LEON K. JONES

(Accepted for publication January 15, 1940)

A leaf-spot disease has been observed on *Sansevieria zeylanica* Willd., and *S. zeylanica* var. *laurentii* Hort. in many of the glasshouses in the State of Washington. Often every leaf on a plant may show roundish, somewhat sunken, reddish-brown lesions, $\frac{1}{2}$ to 1 cm. in diameter, with yellowish borders (Fig. 1). The spots may be limited to one side of the leaf and develop a raised, corky surface. In many cases, the spots extend through the leaf, and

³ Identification of most of the fungi isolated was made by L. M. Fenner, formerly Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry. Transfers of unidentified fungi isolated from the samples were examined by Ross W. Davidson, Associate Mycologist, Division of Forest Pathology, Bureau of Plant Industry.

¹ Published as Scientific Paper No. 429, College of Agriculture and Agricultural Experiment Station, State College of Washington.

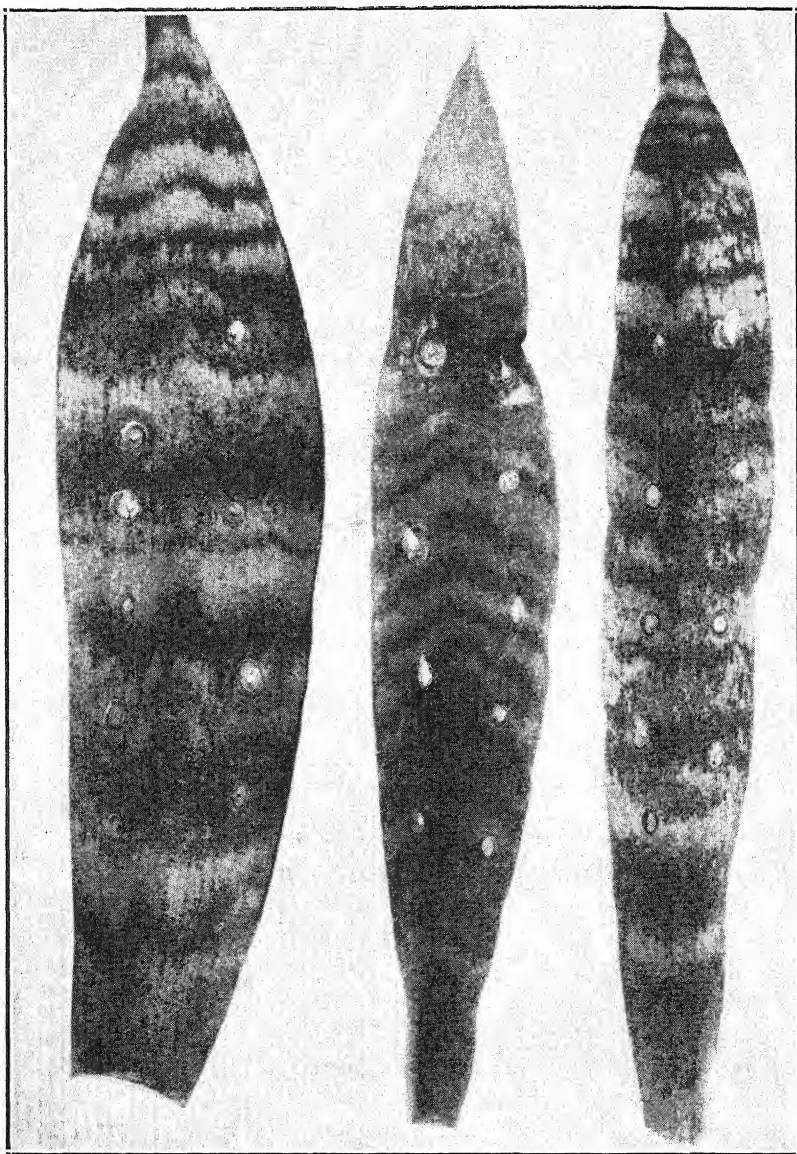


FIG. 1. *Fusarium* leaf spot of *Sansevieria* produced by inoculation in needle punctures with *Fusarium moniliforme*. Center leaf shows girdling by the organism near the tip.

the center dries and falls out. Lesions may sometimes coalesce, encircle the leaf, and cause the death of the distal portion. A similar trouble caused by *Fusarium moniliforme* Sheldon was reported by Kotthoff² as common on *Sansevieria* in Germany.

Isolations on potato-dextrose agar were made from 32 spots on 26 affected

² Kotthoff, P. Neue Topfpflanzenkrankheiten. Kranke Pflanze 14: 28-30. 1937.

leaves, and 20 of the isolates proved to be a species of *Fusarium*. Bacteria were isolated from 5 spots and another species of *Fusarium* from 2 spots. Inoculations with the 3 organisms showed that the predominant isolate was the only one capable of producing the disease (Table 1).

TABLE 1.—Results of inoculations to determine the causal agent of *Sansevieria* leaf spot

Inoculum	Source of organism	Inoculations			
		Inoc. in injuries	Number infections	Not injured	Number infections
<i>Fusarium moniliforme</i> Sheldon	<i>Sansevieria</i>	87	53	10	6
<i>Fusarium</i> sp.	"	16	0	10	0
<i>Fusarium</i> sp.	Beet	64	0	10	0
Bacterial culture	<i>Sansevieria</i>	26	0	10	0
<i>Fusarium martii</i> App. and Wr. var. <i>pisi</i>	Pea	10	0	10	0
<i>Fusarium conglutinans</i> Woll. var. <i>callistephi</i> Beach	Aster	32	0
<i>Botrytis allii</i> Munn	Onion	42	12
<i>Penicillium expansum</i> Lk. ...	Apple	16	0
<i>Penicillium gladioli</i> L. McC. and Thom	Gladiolus	16	16
<i>Fusarium</i> sp.	Iris	16	0
Sprayed with water	5	0	5	0

It was considered that possibly other fungi might be capable of growing in the succulent leaf tissue of *Sansevieria* and, accordingly, a number of common species were used as inoculum (Table 1). The results of these inoculations show that *Botrytis allii* and *Penicillium gladioli* were capable of producing lesions $\frac{1}{2}$ to 1 cm. in diameter following inoculation into needle-punctured areas. *B. allii* and *P. gladioli* produced circular, water-soaked sunken lesions, those of the former being greenish-brown, the latter greyish-green. The lesions caused by *B. allii* were dried and inactive 14 days after removal from the moist chamber, but the *P. gladioli* lesions were still enlarging after 14 days, and in one case continued and girdled the leaf near the point of inoculation.

The *Fusarium* sp., predominately isolated from *Sansevieria* leaf spot and proved to be the causal agent by inoculation and reisolation, has been determined to be *Fusarium moniliforme* Sheldon.³

Infection of *Sansevieria* in injuries made by pricking the leaf with a needle was readily obtained with *F. moniliforme*. Also a number of lesions appeared in uninjured tissue at the base of the leaves where a moisture-holding cup is often formed (Fig. 2). The lesions at the base of the leaves often girdled the leaves and caused the upper portion to die.

It has been recommended that a 4-6-50 Burgundy mixture be used in repeated treatments for the control of this disease. Tests with 4-6-50 Burgundy mixture plus $\frac{1}{2}$ per cent Penetrol proved to be satisfactory as regards

³ Acknowledgment is due Dr. C. D. Sherbakoff for verification of determination.

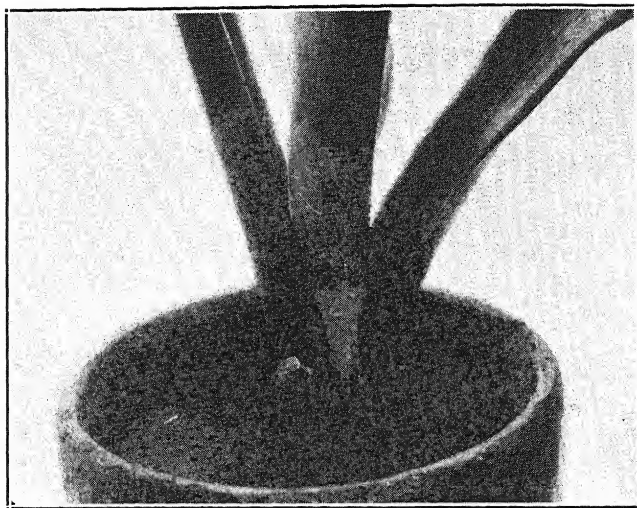


FIG. 2. Infection of Sansevieria leaf near the base by *Fusarium moniliforme* following inoculation of uninjured leaves.

coverage and lack of host injury. Sanitary practices in destroying diseased leaves and care in watering so that spores are not washed about on wet leaves should aid in reducing infection.

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A PINK STAIN OF WOOD CAUSED BY A SPECIES OF GEOTRICHUM

MAE SPRADLING CHIDESTER
(Accepted for publication February 16, 1940)

INTRODUCTION

A jasper pink¹ or light jasper red stain first attracted my attention when some southern yellow pine lumber was received from New Orleans, Louisiana. This lumber was infected with *Fomes pini* (Thore) Lloyd and the stained wood bordered the decayed portion. The material was believed by the consignor to be so-called red heart, a name commonly used for the incipient stage of *F. pini* rot. However, the color was distinctly different from that of red heart. Moreover, it not only bordered the *F. pini* decayed heartwood but extended out from the heartwood into the sapwood. Hubert² described a somewhat similar stain occurring in box elder trees, but no re-

¹ Ridgway, Robert. Color standards and color nomenclature. 43 pp., illus. (Washington.) 1912.

² Hubert, E. E. The red stain in the wood of box elder. Jour. Agr. Res. [U.S.] 26: 447-457. 1923.

port of such a stain in pine could be found. Hedgecock³ reported *Penicillium aureum* Corda and two other species of *Penicillium* as capable of staining pine wood orange red to crimson red, and *Fusarium roseum* Link as the fungus causing pink, red, or violet blotches in pine lumber. Scheffer and Lindgren⁴ reported *Fusarium moniliforme* Sheld. as the cause of pink patches in the sapwood of southern yellow pine. Since apparently similarly stained pine had not been described, it was considered desirable to identify the causal agent.

CAUSAL FUNGUS

A mold was isolated from the pink-stained specimens. When grown at room temperature (about 25° C.) on malt medium⁵ the fungus becomes mealy in appearance in a few days, because of numerous clumps of spores that vary *en masse* from an ivory yellow to a baryta yellow. The malt medium, on which the fungus grows rapidly, soon becomes pinkish. The under side of cultures becomes dark-specked, due to certain portions of the mycelium turning dark brown (Pl. II, A). Some of the mycelium turns a tyrian blue.

The conidia are borne in chains formed by the divisions of the branches of the much and irregularly branched conidiophores (Pl. II, B). The mature spores are hyaline, short cylindrical (Pl. II, C), extreme range 2 μ to 3.6 μ by 2.7 μ to 4.1 μ and sextile range 2.7 μ to 3.4 μ by 3 μ to 3.7 μ . The hyphae vary considerably in size, being from 2 μ to 13 μ in diameter.

The fungus was referred to the genus *Geotrichum* by Diehl.⁶ From a preliminary comparison with descriptions of species of this genus it seems to be a new species, but because of the confusion in the mycological literature concerned with *Geotrichum* and related genera no further attempt was made to classify the fungus or describe it as new.

The author has isolated the same fungus from red-stained cypress heartwood lumber, and Davidson⁷ has obtained it from pink-stained heartwood of a decaying oak log.

THE STAINING ABILITY OF GEOTRICHUM SP.

The staining ability of *Geotrichum* sp. was tested on the heartwood and sapwood of 8 different species of wood. Cultures originating from a single spore⁸ of *Geotrichum* sp. isolated from southern yellow pine heartwood were used to inoculate 10 $1 \times \frac{1}{2} \times 10$ in. sticks, 5 of which were sapwood and 5 heartwood, of each of the following species of wood: silver fir (*Abies ama-*

³ Hedgecock, G. G. Studies upon some chromogenic fungi which discolor wood. Mo. Bot. Gard. 17th Ann. Rpt. (1906): 59-114. 1906.

⁴ Scheffer, T. C., and R. M. Lindgren. Some minor stains of southern pine and hardwood lumber and logs. Jour. Agr. Res. [U.S.] 45: 233-237. 1932.

⁵ Trommer's plain malt extract 25 gm.

Bacto-agar 15 gm.

Distilled water 1000 cc.

⁶ Diehl, W. W., Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

⁷ Davidson, R. W., Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

⁸ Single-spore isolations made by H. C. Greene of the University of Wisconsin.

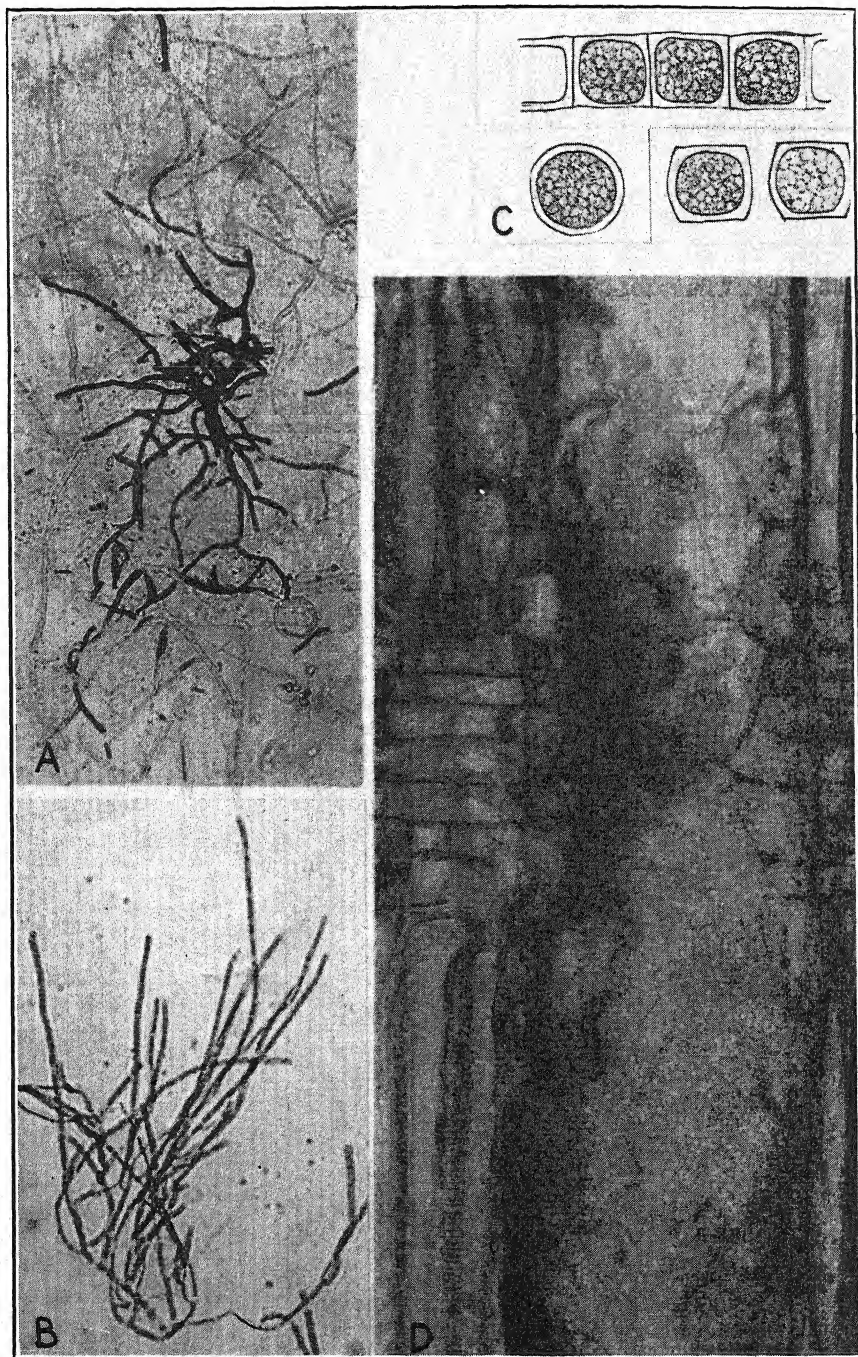


PLATE II. A. Mycelium grown on malt agar. $\times 300$. B. Conidiophores. $\times 300$. C. Spores. Highly magnified. D. Mycelium in wood. $\times 225$.

bilis (Loudon) Forbes), yellow birch (*Betula lutea* Michaux), black spruce (*Picea mariana* (Miller) Britton, Sterns, and Poggenberg), loblolly pine (*Pinus taeda* L.), Douglas fir (*Pseudotsuga taxifolia* (LaMarck) Britton), red oak (*Quercus borealis* Michaux f.), southern cypress (*Taxodium distichum* (L.) Richard), and western hemlock (*Tsuga heterophylla* (Rafinesque) Sargent). Each stick was steamed 30 minutes at atmospheric pressure prior to inoculation. Within 3 weeks after inoculation all of the sticks were stained from a jasper pink to a light jasper red, the same color as the stained specimens from which the fungus was originally isolated. Sticks with a moisture content of 90 to 100 per cent were stained more intensely and more uniformly than sticks with a moisture content of 40 to 50 per cent. The sticks with high moisture content were stained throughout. Microscopical examinations showed only scattered hyphae (Pl. II, D) throughout the wood, but they were somewhat more numerous in the resin ducts and rays.

SUMMARY

A fungus isolated from pink-stained southern yellow pine sapwood and heartwood that is morphologically like a fungus isolated from the heartwood of cypress and from oak was found to be capable of producing the same color in the heartwood and sapwood of loblolly pine, yellow birch, cypress, western hemlock, black spruce, silver fir, red oak, and Douglas fir. It has been classified as a species of *Geotrichum*.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
IN COOPERATION WITH
THE FOREST PRODUCTS LABORATORY,
U. S. DEPARTMENT OF AGRICULTURE.

THE OCCURRENCE OF HELMINTHOSPORIUM TURCICUM IN THE SEED AND GLUMES OF SUDAN GRASS¹

S. J. P. CHILTON²

(Accepted for publication March 1, 1940)

Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) is severely attacked at times by *Helminthosporium turcicum* Pass. How this fungus, which attacks maize and sorghum, overwinters in colder climates is apparently unknown (2), although Eddins (1) states that in Florida it overwinters on debris in the field. Species of *Helminthosporium* have been isolated from maize seed (3, 6, 7, 8), Valteau (8) demonstrating a high percentage of infected seed when a special technique was used. McDonald (4) obtained no evidence of seed infection by *H. turcicum* from

¹ A contribution from the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States.

² The writer wishes to express his gratitude to the agronomists and seed companies who furnished the seed.

maize crops heavily infected with the fungus. Sherbakoff and Mayer (5), however, report it as causing an ear rot, and A. H. Eddins, in a letter to the writer, states that he has isolated the fungus from maize seed. It is the purpose of this paper to report its presence and prevalence in the seed and glumes of Sudan grass.

MATERIALS AND METHODS

Fifty-two lots of Sudan grass seed produced in 10 States were used in the studies. Twelve lots were produced in 1939, 34 in 1938, 5 in 1937, and 1 in 1936. The lots from the 1936, 1937, and 1938 crops were tested in the spring of 1939, those produced in 1939, in the fall of the same year. The technique for determining the presence of fungi consisted in separating the seed and glumes, surface sterilization with 95 per cent ethyl alcohol for one minute, 1-1000 aqueous solution of bichloride of mercury (5 minutes for seed and 1 minute for glumes), followed by immersion in a saturated solution of calcium hypochlorite until transferred to plates of potato-dextrose agar. Both seed and glumes were left from 6 to 15 days, after which identifications were made. One hundred and fifty or more seeds and 88 to 200 glumes were tested for each lot. Over 9,000 seeds and 5,000 glumes were plated. In the case of *Helminthosporium turcicum*, inoculations with appropriate checks were made with single-spore cultures on Sudan grass plants and the fungus reisolated to ensure pathogenicity of the isolates. Germination studies of the seed were made on moistened filter paper in sterile Petri dishes.

EXPERIMENTAL RESULTS

The data in table 1 show that 21 of the 52 seed lots, or 40 per cent, were infected with *Helminthosporium turcicum*, and one or more infected lots were obtained from 7 of the 10 States. The fungus was demonstrated in the seed and glumes of 16 lots, in the glumes of 3 lots, and in the seed alone in 2 lots. The percentage of infected seed varied from 1 per cent to 20 per cent,

TABLE 1.—Seed lots of Sudan grass from which *Helminthosporium turcicum* was isolated at State College, Pennsylvania, in 1939

Source of seed	Number lots tested	Number lots infected	Number lots with seed and glumes infected	Number lots with glumes alone infected	Number lots with seed alone infected
California	11	0			
Georgia	2	1	1		
Illinois	5	5	3	2	
Kansas	2	0			
Nebraska	15	9	8	0	1
New Mexico	2	1	1		
Oklahoma	2	2	1	1	
South Dakota	2	1	1		
Texas	10	2	1		1
Wisconsin	1	0			
	52	21	16	3	2

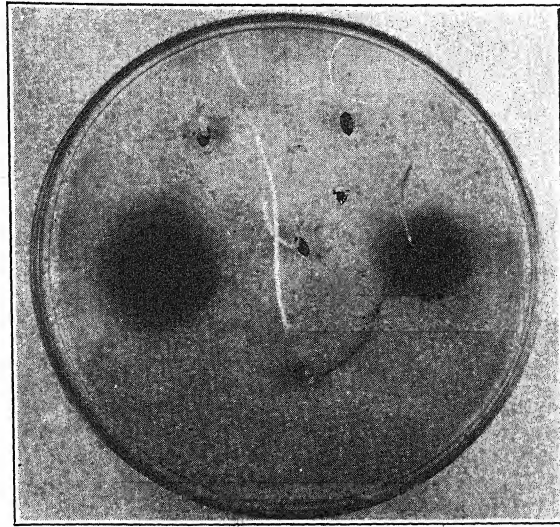


FIG. 1. *Helminthosporium turcicum* emerging from surface sterilized seed of Sudan grass stored two winters.

and of glumes from 1 per cent to over 50 per cent, respectively (Table 2). It is possible that the fungus was present in the other seed lots and was superficial enough to be destroyed by the sterilization technique, or that a random sample was not secured. The fungus was found in the seed or glumes of 4 of the 5 seed lots produced in 1937, indicating that the fungus can remain viable in seed and glumes stored 2 winters. As high as 8 per cent of the seed and 3 per cent of the glumes of these lots yielded *Helminthosporium turcicum*. Figure 1 shows the fungus emerging from seed nearly 2 years old.

TABLE 2.—Frequency distribution of seed lots of Sudan grass with respect to percentage infection of seed and of glumes with *Helminthosporium turcicum*

Materials	Per cent					
	1-5	5.1-10	10.1-15	15.1-20	20.1-50	Over 50
Seed	8	5	3	2	0	0
Glumes	10	5	1	1	1	1

Seed of 2 infected lots was sterilized for 5 minutes, 1 hour, and 3 hours, respectively, in bichloride of mercury solution, the time in alcohol and calcium hypochlorite being the same as previously mentioned. The results (Table 3) show that 1-hour sterilization reduced germination from 90.4 per cent to 87.9 per cent and the percentage of seed from which *Helminthosporium turcicum* emerged from 15.2 per cent to 2.7 per cent. Three hours' sterilization reduced germination to 72.7 per cent and seed from which the fungus emerged to 1.2 per cent. These results indicate the fungus was located primarily, if not wholly, in the seed coat.

TABLE 3.—Emergence of *Helminthosporium turcicum* and germination of seed from 2 lots of Sudan grass seed after different periods of sterilization

Lot	Sterilization time in bichloride of mercury								
	5 minutes			1 hour			3 hours		
	Number seeds	Percentage germination	Percentage infected	Number seeds	Percentage germination	Percentage infected	Number seeds	Percentage germination	Percentage infected
1	202	92.6	18.8	205	91.2	2.9	224	73.7	2.2
2	205	88.3	11.7	201	84.0	2.5	204	71.6	0.0
All	407	90.4	15.2	406	87.9	2.7	428	72.7	1.2

Germination tests of 22 seed lots free of *Helminthosporium turcicum* and 13 infected lots indicate that somewhat fewer seeds germinated in infected lots than free ones, the average germination being 83.5 per cent and 75.5 per cent, respectively. The difference is statistically significant.

Other fungi isolated were species of *Alternaria*, *Helminthosporium*, *Acrothecium*, *Oospora*, *Penicillium*, *Fusarium*, *Chaetomium*, and *Phoma*. As high as 70 per cent of the seeds and 52 per cent of the glumes of some lots were found to contain *Alternaria* spp. *Colletotrichum graminicolum* (Ces.) Wilson was found once or twice in several seed lots and in over 50 per cent of the seeds and glumes of one lot from Georgia.

SUMMARY

Helminthosporium turcicum is reported as occurring in the seed and glumes of a high percentage of the Sudan grass seed lots tested. The fungus may remain viable at least 2 winters in both seed and glumes. With respect to the seed, infection is confined primarily, if not wholly, to the seed coat. Germination was reduced somewhat in the case of infected seed in the lots tested.

U. S. REGIONAL PASTURE RESEARCH LABORATORY,
DIVISION OF FORAGE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY,
STATE COLLEGE, PA.

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PHYTOPATHOLOGICAL NOTES

Monilinia Causing a Brown Rot and Blight of the Common Azalea.—In a previous paper¹ brief reference has been made to a species of *Monilinia*, which is parasitic and pathogenic upon the common azalea or pinxter flower, *Rhododendron roseum* Rehder (= *Rhododendron nudiflorum* (L.) Torr. var. *roseum* (Loisel.) Wiegand). The name *Monilinia Azaleae* has been proposed for this fungus.

The purpose of the present note is to present a technical description of this fungus for reference, pending the appearance of a more extended paper that has been prepared for publication.

The apothecial stage arising from the mummied, overwintered fruits or capsules of the host was collected on the ground beneath the host plant by the writer on various field trips in May 1924, April and May 1925, and May 1927, at several locations near Ithaca, New York.

The monilioid conidial stage appeared upon leaves and young succulent shoots when the host plant was in bloom early in June and the young pseudosclerotia and the conidial stage within and upon the young developing fruits during the latter part of June and in July in the same locations.

Collections of a monilioid conidial stage causing a leaf blight of *Rhododendron canescens* (Michx.) G. Don made at Winterville near Athens, Georgia, in April 1925, by Dr. Julian Miller, and later examined by the writer, appear to be of the same species.

The measurements used here are for fresh living material.

Monilinia azaleae, sp. nov.

Apothecia 1-2 crescentia parva fundamenta ab exteriori pseudosclerotii in munitis fructibus, adulta, 0.83-3.5 cm. in altitudine, stipitata, cyathiformia ad patelliformia, discus cinnamon-brown (R.)² ad Prout's brown (R.) ad obscuriorem fuscum-atrum, vertens omnino ad atrum ad inferiorem dimidium stiptis et in rhizoideo caessite stipes laevis et gracilis, cylindraceus, attenuatus leviter et puberulus ad inferiorem partem 0.5-2.0 mm. latitudine et 0.4-3.0 cm. longitudine; rhizoideus caespes praesens, conspicuus, atrofuscus, capilliform, flabelliform radians, ex inferiore parte stipitis; discus aperiens cyathoid et tum infundibuliformis, postea patelliformis 0.2-1.4 cm. diam., margo exilis interdum maturus fissuratus et recurvus; asci cylindracei clavati 178-258 \times 11-16.5 μ , modus 211.5 \times 13.2 μ medianus 213.48 \times 13.8 μ apice rotundo incrassato perforato poro, cuius claudens substantia ope jodi aliquanto cyanescens tingit, octospori; ascospori saepe obliqui et uniseriales in parte superiore asci, saepe subbiserialis, elliptici finibus rotundis, hyalini, continui, saepe cum centrali refringente macula, limites 9-20 \times 5-14 μ , modus 15 \times 8.75; medianus 13.88 \times 9.48 μ ; paraphyses abundantes, filiformes, ascis aequilongi, apicibus subclavatis attenuati paulatim ad basem, continui vel uno aut duobus septis ad partem inferiorem, hyalini; ectostroma crescens sub epidermem, maxime in foliis, juvenilibus ramulis et fructibus fructificans cinereum incrementum de conidiis, saepe nervicola in superficie superiore foli et in superficie fructus; conidia (macroconidia) limoniformia continua, hyalina, limites 8.5-19.0 \times 5.5-14.5 μ , modus 11.1 \times 8.8 μ , medianus 12.37 \times 9.59 μ , crescens in longis di-et trichotomis catenis, adultum parvi, fusiformes disjunctores saepe inter conidia; spermatia (microconidia) non observata; pseudosclerotia in aegrotantibus capsulis, adulta complementia loculos fructos hyalinis hyphis incrassatis muris dispositis paliforme in contactu cum vallo pericarpii et dissepimentorum placenta et ovules circum data in albo incremento fungi, solidum, complens

¹ Honey, E. E. North American species of *Monilinia*. I. Occurrence, grouping and life histories. Amer. Jour. Bot. 23: 101, 105, 106. 1936.

² (R.) = Ridgway, R. Color standards and color nomenclature. 43 pp., 53 col. pl. Washington, D. C. 1912.

mumificatam capsulam; hab., parasitica in foliis, ramulis et fructibus *Rhododendron roseum* Rehder, Enfield Gorge, Ithaca, Tompkins County, New York.

Apothecia one to two arising as small fundaments from the outer surface of the pseudosclerotium in mummied fruits, attaining at maturity a height of from 0.83–3.5 cm., stipitate, cyathoid to patelliform, disc cinnamon-brown (R.) to Prout's brown (R.) to a darker brown-black, becoming entirely black toward the lower half of the stipe and on the rhizoidal-tuft; *stipe*, smooth, slender, cylindrical, tapering slightly and somewhat pubescent toward the lower portion, 0.5–2.0 mm. in breadth and 0.4 to 3 cm. in length; *rhizoidal-tuft* present, conspicuous, blackish, capilliform, radiating, somewhat fan-shape from its point of origin on the basal portion of the stipe; *disc* expanding becomes cyathoid, then infundibuliform, later patelliform, from 0.2 to 1.4 cm. in diameter; margin then occasionally cleft at maturity because of a recurving of the disc resulting in radial splitting from the circumference inward;

Asci cylindric-clavate, 178–258 \times 11–16.5 μ , mode 211.5 \times 13.2 μ , mean 213.48 \times 13.8 μ with rounded thickened apex perforated by a pore, the closing substance of which stains moderately blue with iodine, 8-spored; *ascospores* commonly arranged obliquely uniseriately in the upper end of the ascus or occasionally subseriately, elliptical, with rounded ends, hyaline, continuous, commonly with a characteristic-shape central refractive spot, measurements give limits of 9–20 \times 5–14 μ , mode 15 \times 8.75, mean 13.88 \times 9.48 μ ; *paraphyses* abundant, filiform, about the same length as the asci, slightly swollen toward the tips, gradually tapering toward the base, nonseptate or with 1 or 2 septa toward the basal region, hyaline.

Ectostroma developed beneath the epidermis, particularly on the leaves, the young succulent shoots and fruits, forming an ash-gray coating of the conidial fructification, commonly on the upper surface of the midrib of the leaf and on the surface of the fruits.

Conidia (macroconidia) limoniform, continuous hyaline, measurements give limits of 8.5–19 \times 5.5–14.5 μ , mode 11.1 \times 8.8 μ , mean 12.37 \times 9.59 μ , borne in long di- and trichotomously branched chains, at maturity small fusiform disjunctors commonly present between the conidia.

Spermatia (microconidia) not observed but possibly present in this species.

Pseudosclerotia develop in the infected capsules, at maturity, filling the loculi of the immature fruit with a solid mass of thick-walled hyaline hyphae that assumes a more or less palisade-like arrangement at the point of contact with the wall of the pericarp and the dissepiments, the browned and shrivelled remains of the dissepiments, placenta, and the ovals are plainly recognizable embedded in the white fungus growth of the solid pseudosclerotium in the mummied capsule in cross sections, capsules containing pseudosclerotia do not open but fall to the ground, overwinter, and later may give rise to the apothecia.

Host. The apothecial stage develops on the fallen mummied fruits of the common azalea, *Rhododendron roseum* Rehder, which have overwintered on the ground in the leaf mold under the shrubs in somewhat moist shaded places during the latter part of April and the first part of May in central New York.

The conidial stage is parasitic upon *Rhododendron roseum* appearing first upon scattering leaves and young succulent shoots when the host is in full bloom, early in June, and common upon the young and developing fruits the latter part of June and July.—

EDWIN E. HONEY, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

*Fruit Stripe of Tomato Caused by a Tobacco Type 1 Virus.*¹—During the summers of 1936 and 1937, some of the tomato fruit harvested from the experimental field plots at Pullman, Washington, showed chlorotic to necrotic stripes extending from the stem end towards the blossom end (Fig. 1). Cuttings were made from the affected plants and transferred to the glasshouse for study. Transfers from the affected cuttings to benched tomato plants in a comparative test with transfers of tobacco mosaic, potato vein-banding, potato mottle, and combinations of these diseases showed the tomato fruit stripe to be distinctive in symptoms.

SYMPTOMS

The distinguishing symptoms of the disease are the stripes on the fruit. They appear first near the stem end as somewhat raised light-green to

¹ Published as Scientific Paper No. 430, College of Agriculture and Agricultural Experiment Station, Pullman, Wash., D. C.

ashen-grey stripes, 1 to 2 mm. in width, radiating from the point of stem attachment for varying distances towards the blossom end of the fruit. As the fruit enlarges the stripes may become broken, somewhat brownish, and sunken (Fig. 1).

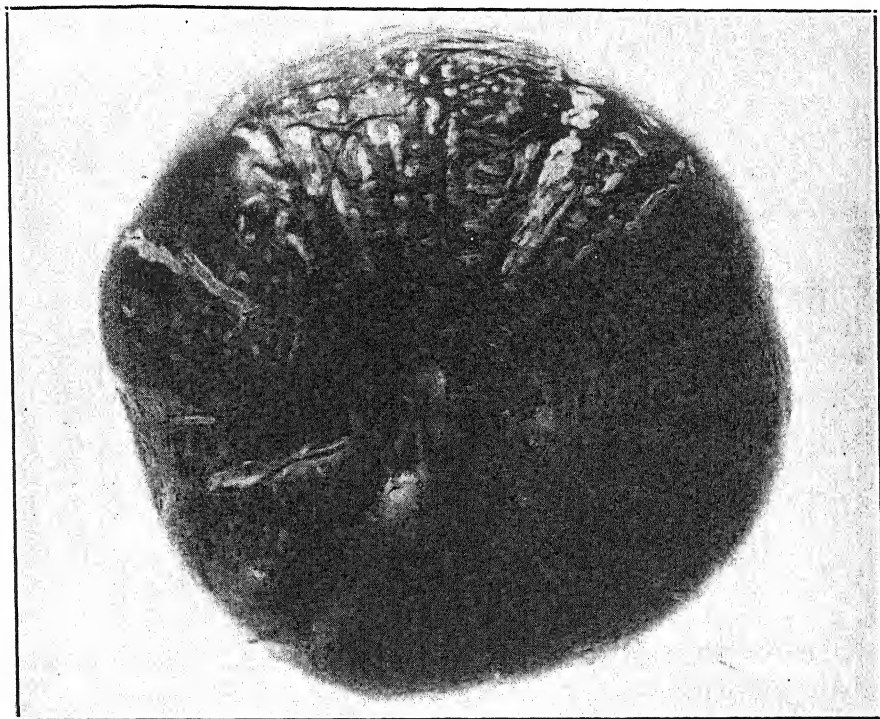


FIG. 1. Tomato fruit showing chlorotic and necrotic stripes radiating from the stem end.

The foliage of affected plants shows a mild mosaic (Fig. 2, C) without necrosis. No necrosis is noted in stems or petioles, as is the case with plants affected with the single virus streak, or the streak disease caused by the combination of the tobacco-mosaic virus and the potato X virus. On tobacco foliage the virus produces only a mild mosaic on the young leaves (Fig. 2, B), which is followed by numerous small chlorotic spots as the leaves grow older (Fig. 2, A).

Combination of the potato X virus and the fruit-stripe virus on tomato plants produced streak symptoms, and on tobacco plants leaf-necrosis symptoms similar to those produced by combining the tobacco mosaic virus and the potato virus in these susceptibles.

CHARACTERISTICS OF THE VIRUS

Inoculations to 10 tomato and 10 tobacco plants in each test showed the fruit-stripe virus to have an incubation period of 12 to 15 days; it was

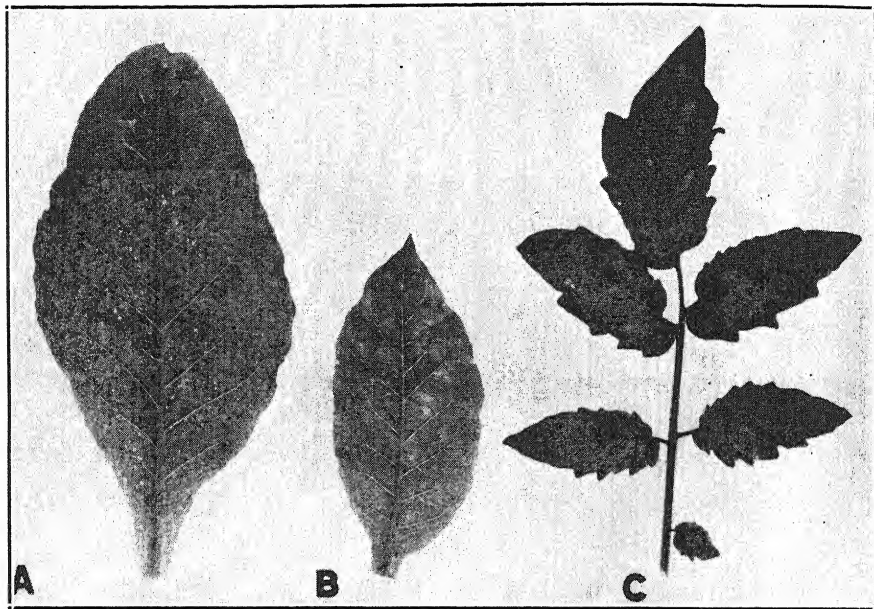


FIG. 2. Effect of tomato fruit-stripe virus. A. Older tobacco leaf. B. Young tobacco leaf. C. Tomato leaf.

inactivated at 90° C. and not at 80° C. for 10-minute exposures; it remained active in 1:1,000,000 dilution with water; and remained active for a period of at least 65 days *in vitro*. The general characteristics of the virus were the same as those exhibited by tobacco virus 1 and, accordingly, it is considered a variant of the tobacco-mosaic virus.—LEON K. JONES, Division of Plant Pathology, Agricultural Experiment Station, The State College of Washington, Pullman, Washington.

*Fumigation Injury of Chrysanthemum.*¹—It is a common practice in glasshouses to burn Nico-fume powder for the control of aphids. During the growing season, of 26 varieties of chrysanthemums, in a study of the *Verticillium* disease, the house was fumigated with Nico-fume when the buds of many varieties were partially opened. Nico-fume, to fill two 2½-in.

TABLE 1.—*Chrysanthemum* varieties subjected to Nico-fume fumigation

Adrian's Pride	Early Frost	October Frost
Ambassador	Friendly Rival	Pink Mistletoe
Bonaffan	Golden Measure	Pink Treasure
Chadwick	Indianola	Razor
Chieftain	Justrite	Seidewitz
December Beauty	Lustre	Turner
December Glory	Maude Dean	Whittier
Dorothy Turner	Monument	W. H. Waite
Dr. Enguehardt	Oconto	

¹ Published as Scientific Paper No. 431, College of Agriculture and Agricultural Experiment Station, State College of Washington.

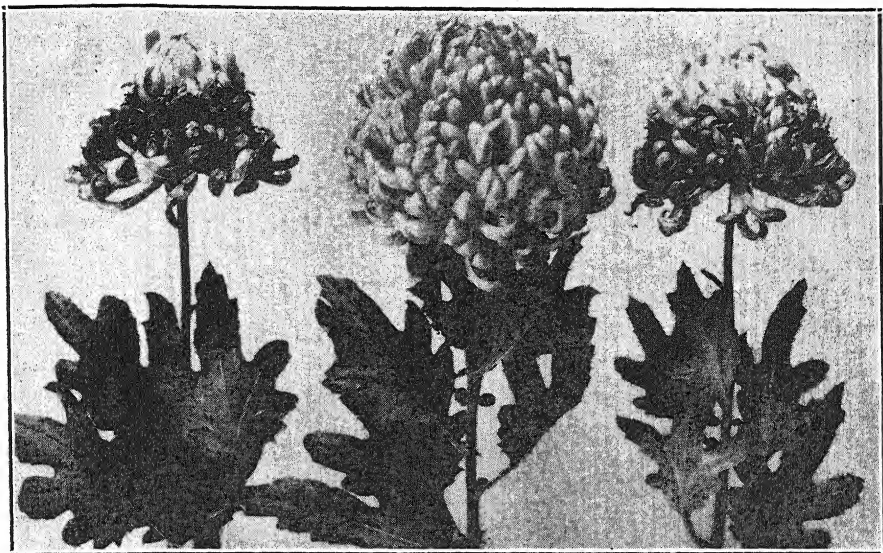


FIG. 1. Ring of necrotic floral parts of Whittier blossoms caused by Nico-fume fumigation. Uninjured blossom in center.

pots, was burned in 5500 cu. ft. of glasshouse space in the early evening, and the ventilators remained closed until 7:30 the next morning.

When the Whittier variety opened it was noted that most blossoms showed a ring of brown, dead, floral parts, 2 to 3 inches wide near the base (Fig. 1). None of the flowers of the other 25 varieties (Table 1) showed this injury. A similar planting of the 26 varieties in an adjacent section of the glasshouse was not fumigated and all blossoms were free from the trouble.—LEON K. JONES, Division of Plant Pathology, Agricultural Experiment Station, The State College of Washington, Pullman, Washington.

*Notes on Septoria Scalds of Vetch and Peas in Oregon.*¹—One of the most important diseases of vetch, particularly *Vicia sativa*, which is an increasingly valuable seed crop in Oregon, is a stem rot or scald and leaf spot caused by *Septoria viciae* West. This fungus causes a purple to vinaceous cortical rot on the lower culm. Spotting and speckling extends up the stems and onto the leaves. After rains in late winter, the spotting coalesces to cause extensive scorching. While the culm injury is confined to cortical cells, the area covered is so extensive that injury is severe and reduction in seed yield is apparent at harvest. Often the fungus does not fruit, making identification uncertain, but in some cases spores are produced in great numbers. Spread of the disease is by spores, which, splashed by rains, cause the severe speckling or scalding.

¹ Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published as Technical Paper No. 331 of the Oregon Agricultural Experiment Station with the approval of the Director. Contribution from the Department of Botany.

The fungus has two kinds of pycnospores, macrospores ($53-71 \times 1.7-2.1 \mu$) and microspores ($3.5-11 \times 1.2-1.5 \mu$) (Fig. 1). The macrospores are

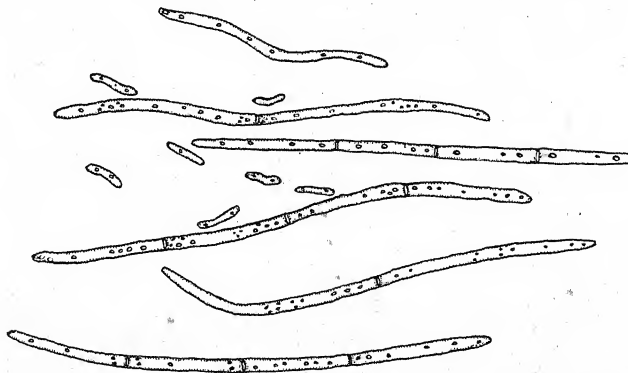


FIG. 1. Pycnospores (macrospores and microspores) of *Septoria viciae* West. on *Vicia sativa*. Granger, Oregon, June 13, 1939. $\times 1000$.

straight to curved or slightly sinuous, broadly filiform, mostly 3-septate, with small oil drops in the cells. The microspores are nonseptate and bacillar-shape. The fungus can be assigned to *Septoria viciae* West. Common vetch is very susceptible, Hungarian vetch apparently less so.

A cortical stem scald of Austrian field peas has been observed in Oregon for a number of years. Its symptoms are similar to those of *Septoria* scald on vetch. It apparently is caused by *Septoria pisi* West and, in Western Oregon, is often the sole cause of severe injury to Austrian field peas in early spring. In other cases the fungus occurs with *Ascochyta*.

These diseases should be studied further as they are of economic importance, particularly in the control of seed-crop diseases in Oregon.—
RODERICK SPRAGUE, North Dakota College of Agriculture, Fargo, N. D.

BOOK REVIEW

THOMPSON, HOMER C. *Vegetable Crops*. (3rd ed.) 578 pp., 68 figs., \$5.00. McGraw-Hill Book Company, New York and London. 1939.

Although this textbook was prepared primarily for students and others interested mainly in the culture, production, and economics of vegetables, phytopathologists and economic botanists will find it especially valuable as a reference. In it the author, who has spent his entire career specializing in this particular field, has assembled a veritable mine of scientific data on the different phases of the vegetable industry.

The book consists of 27 chapters and is well illustrated with 68 figures, besides numerous tables. It might be said really to be composed of two parts. The first part consists of 15 chapters in which the author discusses the scientific facts and principles involved in the growth of the vegetable from germination of its seed to its harvest and storage or placement on the market. There is treated in these chapters such pertinent subjects as soils and soil preparation, manures, soil-improving crops, seed and seed growing, and methods for the control of diseases and insects. Information, useful not only to the pathologist specializing in the diseases of vegetables, but also to the extension pathologist or, even the general pathologist called upon to diagnose diseases of vegetables.

The second part of the book is composed of 12 chapters in which the various groups of vegetables are discussed individually. For example, in the chapter devoted to perennial crops, such vegetables as asparagus, artichoke, Jerusalem artichoke (Girasole), and sea kale are considered. In another chapter, the potherbs or greens, spinach, New Zealand spinach, orach, chard, kale, mustard, collards, and dandelions are considered. Other chapters are devoted to cole crops, root crops, bulb crops, and the potato, the sweet potato, beans and peas, solanaceous fruits, the cucurbits or vine crops, and sweet corn.

For each vegetable (at least the important ones) there is given and discussed its history, taxonomy, characteristics, climatic requirements, soil preferences, soil reaction, culture, diseases, and insects, if they are of economic importance.

In this book the author shows that he appreciates the economic importance of diseases and insects in the growing of vegetables, inasmuch as he advises students specializing in olericulture, to schedule during their collegiate careers courses on plant diseases and harmful insects. Furthermore, he stresses the danger of the transmission of diseases and insects into new areas by the indiscriminate shipment of plants.

For each crop the author lists the important diseases and standard control measures. This information assembled under one cover should be especially helpful to vegetable growers who use this book as a reference and are not specialists in the field of phytopathology. Therefore, it is contended that the control measures recommended should be as up-to-date and concise as possible, so as not to be in any way confusing. In some instances, the directions given for controlling some of the diseases are rather incomplete and indefinite or are cited as being effective in a certain section of the country. The user of this book is undoubtedly not so much interested in the effectiveness of treatment in one section as he is in knowing whether he can control or eradicate a disease in his own locality.

In so comprehensive a volume occasional errors and omissions would be expected. In the chapter on the diseases of sweet potatoes the author omits mention of such diseases as soil rot or pox. This disease, which is widely distributed and important, has become very serious in some sweet-potato areas in recent years, so bad in some cases that the yield has been reduced considerably. In some instances it has resulted in the abandonment of any further culture of this vegetable. Reference in the text is made to the monographic study of the diseases of sweet potato by Harter and Weimer but the title or a citation of it is not to be found in the literature cited at the rear of this book.

Inconsistencies appear in the use of authorities for names of seed plants, fungi and insects. Although these are not particularly serious, they may be confusing to the students and others who use this textbook as a reference. For example, the scientific name and authority for cress or garden cress (*Lepidium sativum* L.) is cited, while the authority for the scientific name of chervil or salad chervil (*Anthriscus cerefolium*) occurring on the same page as the preceding vegetable, is omitted. Similarly, some inconsistencies appear in respect to capitalization of specific names. Other small errors and omissions occur throughout the book, but the author will doubtless correct them in his next revision.

—THEODORE T. AYERS, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

ATTENTION! PLANT PATHOLOGISTS

Your committee on Publicity and Public Relations has been charged with the duty of working out ways and means of bringing before the public, as soon as it becomes available, information concerning the newer developments in the nature and control of plant diseases.

The functions of the committee obviously cannot be carried out without the continued cooperation of the members of the Society. The committee earnestly appeals to all active plant pathologists to send in newsworthy material of their work. These may be reprints of new publications, a copy of the manuscript after acceptance by a scientific journal, or a description of the work designed especially for the committee's use.

From this information, the committee will develop news articles or magazine articles or will transmit the information to one of the several science writers to be syndicated in their publications. In every case the author will be given full credit. The information will not be used without the full consent of the author and of the authorities at his institution, if such consent be necessary. It will be the constant care of the committee that no untrue or distorted interpretation of the facts shall be written by reporters.

The committee will not interfere in any way with any established State, federal or other publicity work.

We would suggest that all available information be sent to the committeeman representing your section of the country or to the chairman if you so desire. Please state if the information can be used immediately or give its approximate date of release.

C. T. GREGORY, *Chairman*

Committee on Publicity and Public Relations

SUMMER MEETING OF THE NORTH CENTRAL STATES GROUP OF PHYTOPATHOLOGISTS

The North Central States group of phytopathologists will conduct a summer tour in western Illinois from June 20 to 22. The group will assemble at Quincy, Illinois, on June 20. June 21 will be spent on tree fruit and small fruit diseases near Quincy and on grain diseases in the Illinois River bottom near Jacksonville. The afternoon will be devoted to an inspection of the experimental orchard spraying work at Jerseyville. The night will be spent at the famous Pere Marquette State Park near Grafton.

On June 22, the group will tour the intensive vegetable area in the Mississippi River bottom near East St. Louis where various vegetable and field crop diseases will be seen.

Members of the Society other than those in the North Central States (Michigan, Wisconsin, Minnesota, Iowa, Nebraska, Missouri, Illinois, Indiana and Ohio) who plan to attend the meeting should write Dr. H. W. Anderson for detailed program about May 20.

Committee on Arrangements,

C. M. TUCKER

I. H. MELHUS

H. W. ANDERSON

A DESIGN FOR LABORATORY ASSAY OF FUNGICIDES¹

J. G. HORSFALL,² J. W. HEUBERGER,³ E. G. SHARVELLE,⁴
AND J. M. HAMILTON⁵

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INTRODUCTION

It is evident that a surge towards new fungicides is arising. Accelerated tests urgently are needed for sorting the new materials and for developing others.

During some years past the writers have made use of laboratory tests in their research on fungicides. At the outset, however, two difficulties with laboratory technique arose: (a) results were not reproducible from day to day with the same materials, indicating that adequate mechanical equipment or procedures were not-known; (b) protective value in the field could not be predicted accurately from the laboratory results, indicating that definitive techniques had not yet been devised for that purpose.

Investigations, therefore, were undertaken to refine the existing methods for testing fungicides in the laboratory. Mechanical equipment and procedures were redesigned and it is now possible to obtain reasonably reproducible data from day to day and from worker to worker. Some progress has been made in predicting field performance of fungicides (4), although rapid advance in that work has been delayed until the laboratory assay could be stabilized and reduced to a quantitative basis.

It appears that failure to predict the protective value of fungicides in the field accurately can be ascribed to inability to measure and integrate the 3 factors contributing to the protective value of a spray, *i.e.*, deposition, fungicidal value, and tenacity, where deposition means the amount of material left on the surface after treatment, fungicidal value means the ability of the material to prevent spores from germinating or to prevent their growth,⁶ and tenacity means resistance to weathering.

It is the purpose of this paper to present the technique devised for applying uniform deposits of fungicides and for measuring fungicidal value. Since the techniques for measuring and evaluating tenacity of

¹ The research for this paper was conducted chiefly at the New York State Agricultural Experiment Station, Geneva, N. Y., and completed at the Connecticut Agricultural Experiment Station, New Haven, Conn. It was conducted in cooperation with the Crop Protection Institute.

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⁶ This concept of fungicidal value is to be distinguished on one hand from protective value in the field, which refers to ability to prevent infection, and on the other hand from toxicity, which refers to the inhibiting action of the toxic part of the molecule (cupric copper, for instance) of the material concerned.

spray materials will be published elsewhere (3), the subject of prediction of protective value in the field will not be considered in this paper.

MATERIALS AND TECHNIQUE

The Sprayer

When it became necessary to design a sprayer, it was apparent that too much emphasis had been placed on concentration of fungicide in the spray tank, whether in field or laboratory, and too little on the deposition of the fungicide on the sprayed surface. It is not the amount of the material as it exists in the tank that functions to inhibit spores, it is the amount as it exists on the sprayed surface.

With the emphasis on the spray tank it was important to keep concentration under rigid control. Changing the emphasis to deposition in no way lessens the importance of concentration but it shows that spraying technique is likewise of paramount importance, because errors in spraying may be larger than differences between compounds.

In the field the fungicide may be drifted dry onto the foliage by a duster, using the wind stream as a carrier, or it may be sprayed on, using water as a carrier. This paper deals only with deposition by spraying. In the field the fungicide suspension in water is ejected under pressure from a nozzle. Since it is mechanically difficult to put pressure on the water in a laboratory sprayer, an atomizer was used. Compressed air moving across an orifice draws up the spray fluid, ejects it, and acts as the carrier for the fungicide.

The original sprayer made use of a fixed nozzle, a fixed target, and a sliding shutter to interrupt the spray stream (6). After many trials a deVilbiss No. 15 atomizer was adopted. The target was a glass microscope slide fixed in the middle of the spray stream with a friction clip. The fungicide was weighed for 1000 cc. quantities to fill the spray container and stirred mechanically to prevent settling.

Deposition of spray fluid was obtained by weighing a sprayed tared slide that had been placed immediately in a weighing bottle. Three determinations were made. Knowing the concentration of fungicide in suspension, the deposition per unit area could be determined.

Nozzle

Effect of Diameter. Since the size of the hole in the nozzle affects ejection and deposition, it was necessary to calibrate each new nozzle and to recalibrate the old one as it wore larger with use.

Effect of Air Pressure. It was found that increased air pressure increased the ejection and deposition of spray from a nozzle as shown graphically in figure 1. A sensitive reducing valve, such as No. 7C, made by Foxboro Company, Foxboro, Mass., was installed in the air line to maintain a constant air pressure. A small painter's air compressor provided an inexpensive source of compressed air.

Effect of Height of Lift. The atomizer must lift the spray fluid from a container below the nozzle. The effect of the height of lift upon ejection was studied by placing a graduated cylinder below the nozzle. The time required to lift and eject 10 cc. of liquid from various levels was determined and graphed (Fig. 2). It appears that the height of lift bears an almost straight-line inverse relation to ejection. In practice the height of lift was standardized at 16 cm. by using a large-diameter container always filled to the same level with spray fluid. The small amount of

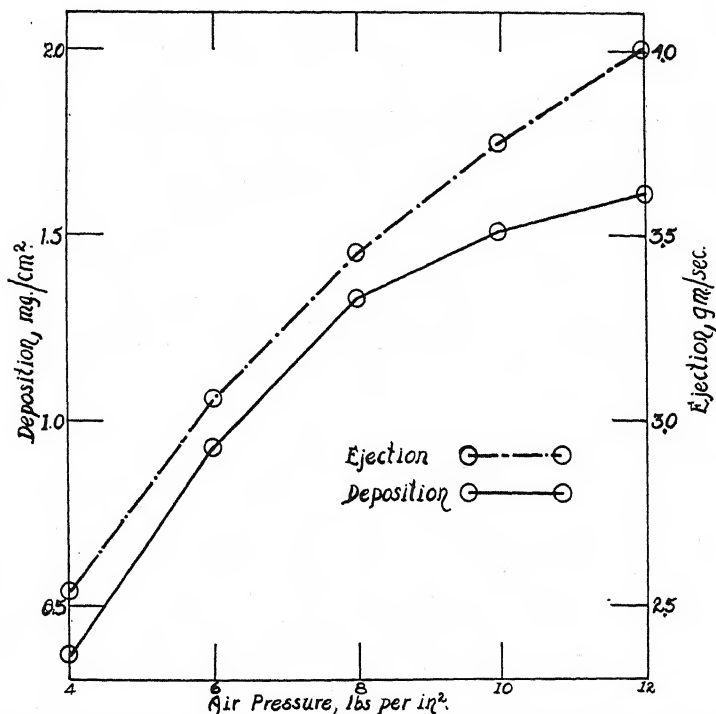


FIG. 1. Effect of air pressure on ejection and deposition from a deVilbiss No. 15 atomizer.

liquid sprayed out in any test did not materially affect the level of the liquid in the container.

Air Stream

Since air served as a carrier stream for the spray droplets bearing the fungicide, laboratory spraying became a two-way problem of air conditioning and aerodynamics.

It became a problem in air conditioning because the spray droplets increased or decreased in size, depending upon the dew point of the air and the vapor pressure of the spray suspension, so that the temperature and humidity of the air through which they pass affect the quantity of the fungicide deposited on the slide. The spray stream was enclosed in a sheet-iron chamber, about 2 feet in diameter, in order to control atmospheric humidity.

A typical dew-point experiment follows: the laboratory air was at 24°C . and 26 per cent relative humidity. The dew-point depression was 20°C ., which means that dew would collect on an object with a temperature of 4°C . ($24^{\circ} - 20^{\circ} = 4^{\circ}$). Water at 8°C . (just above dew point) was sprayed. The deposition of water was $0.368\text{ mg/cm}^2/\text{sec}$. When the hu-

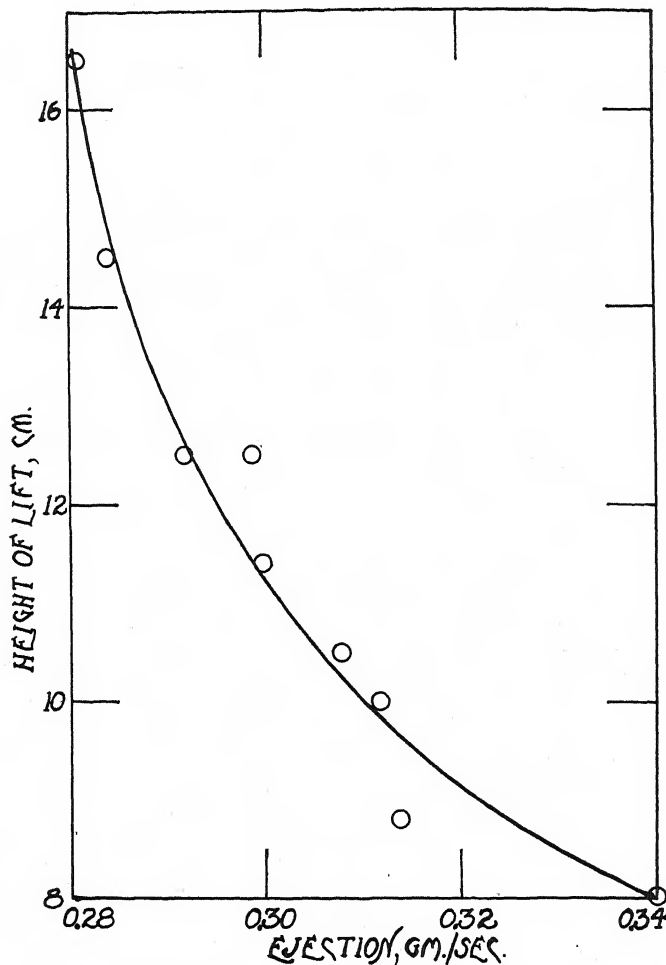


FIG. 2. Effect of height of lift on ejection from a deVilbiss No. 15 atomizer.

midity in the chamber was elevated to 94 per cent by a trickle of steam, the dew point rose to 19.2° , and the deposition to $0.520\text{ mg/cm}^2/\text{sec}$. Thus the cool spray droplets acquired moisture from the humid air while in transit. When the air in the chamber was allowed to return to 26 per cent humidity and the dew point to 4°C ., warm water at 50°C . was sprayed through it. The deposition fell to $0.268\text{ mg/cm}^2/\text{sec}$., because the spray droplets evaporated in transit to the slide.

The question arose as to whether the dew point affects deposition of

fungicide as well as of water. Several tests using the technique described below showed that it does. In a typical test cuprous oxide at 0.1 per cent copper in water at 20° C. was sprayed for 13 and 8 seconds through air at 25° C., first at 30 per cent relative humidity (dew point 7° C.) and later at 90 per cent relative humidity (dew point 23.6° C.). Data (Table 1) indicate that when the dew point was below that of the spray fluid, many of the droplets evaporated, the load of fungicide they carried dropped out, and spore inhibition fell.

TABLE 1.—*Effect of dew point on fungicidal readings, expressed as percentage of spores not germinating*

Spraying time	Dew point temperature	
	7° C.	23.6° C.
<i>Seconds</i>	<i>Per cent</i>	<i>Per cent</i>
13	44.0	50.9
8	15.6	35.0

When the sprayer was enclosed in the large metal case, the problems in aerodynamics increased because the usual eddies at the margins of the spray stream were complicated by back pressure from the end of the chamber. Other efforts having failed to solve the problem, the chamber was reduced to a 4-in. cylinder open at both ends (Fig. 3).

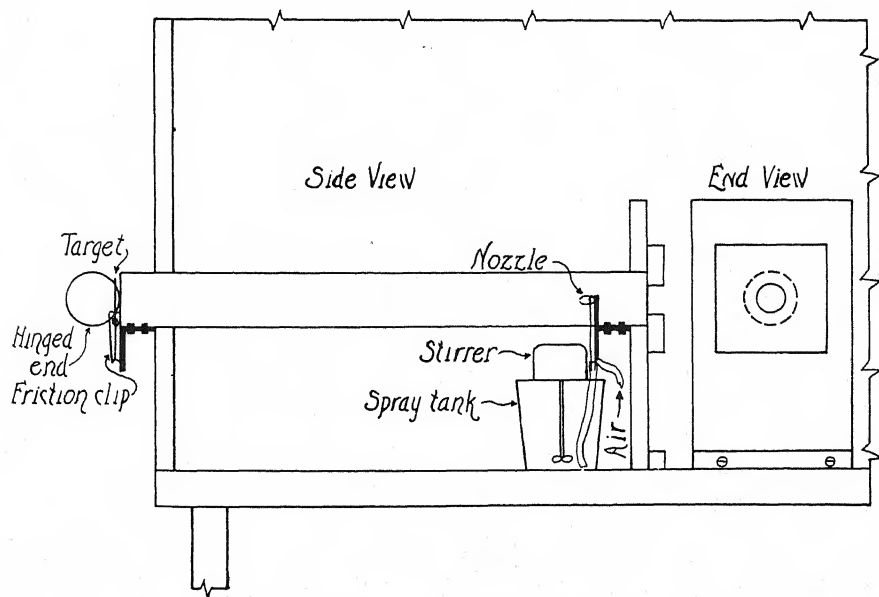


FIG. 3. Sketch of a tube-type precision laboratory sprayer.

The nose guard was removed from the atomizer and the 2 tubes were sawed off close to the bottle connection. The nozzle was then turned at a right angle and the 2 tubes were soldered to a piece of strap iron bent

at a right angle. This was bolted to the bottom of the tube, so that the nozzle was held in the center of the tube about 3 in. from one end and pointing toward the target. A piece of rubber tubing leading to the spray tank was connected to one atomizer tube and the air line was connected to the other.

A 3-in. length of the opposite end of the cylinder was hinged so that it opened. The target slide was held by a friction clip attached to the stationary portion of the tube by a piece of strap iron. A slot was cut in the hinged portion, so that it could be closed over the slide to prevent eddies around it. The slide was 30 in. from the nozzle.

It was found that the size of the droplets on the slide varied with the size of the opening in the nozzle end of the cylinder. The size of this opening was adjusted to give run-off in about 90 seconds of spray time. At short spray exposures, the droplets were exceedingly fine.

Humidity control was obtained by projecting the target end of the tube through the end of a hood where the slides could be positioned without opening the hood. Humidity in the hood was raised with a trickle of steam. A small trap door in the hood door facilitated changing the spray material. This arrangement is shown in figure 3.

McCallan and Wilcoxon (12) report that the day-to-day variation is greater for a horizontal sprayer without dew-point control than for their settling tower. The probable explanation is that the fungicide arrives on the slide in a settling tower even though the carrier droplet may evaporate. Unless dew point be controlled in a horizontal sprayer, some of the fungicide may be lost in transit.

THE SPRAYED SURFACE

Angle of Surface

In this research the target always has been placed vertically so that the spray would not strike it obliquely. This position has the drawback that materials containing spreaders run off so quickly that sufficient deposition may not be reached.

Nature of Surface

The choice of a surface was important because of surface tension. It was, of course, impossible to reproduce either a hirsute or a glabrous leaf surface, but a surface of constant spreading properties was imperative for consistent results. The surface wetting properties of glass seemed to vary, with type of glass, how clean and scratched it was, what cleaner was used, and length of standing after cleaning. When the spreading property varied from time to time the diameter of the spore drop varied, the amount of toxicant per spore varied as discussed below, and the result varied. Cellulose nitrate on glass as suggested by Evans and Martin (1) was adopted as standard. The wetting properties of the film appear to be reproducible from day to day and from laboratory to laboratory. One drop of distilled water when dropped onto this surface from a 1 cc. pipette

held at an angle of 45° and from a height of 1 cm. spreads to 7.5 mm. in diameter in laboratories as far apart as Geneva, New York, New Haven, Connecticut, and Bristol, England. No evidence has been obtained that the cellulose nitrate reacts with spray fluids.

Cellulose nitrate can be purchased under the trade name of Pyroxylin. It should be dissolved to give a 2.5 per cent solution in butyl acetate. The slides were dipped into this solution and dried overnight in a dust-free place before being used.

Spraying Distance

Since deposition varied inversely as the distance of the target from the nozzle, the target was always fixed in relation to the nozzle.

THE DOSAGE

Since the whole range of the fungicidal value of a material should be known, the problem of regulating dosage or amount of deposition arose. Obviously, deposition can be changed quantitatively by changing the concentration of material in the spray tank, or the spraying time. The first is slow because it involves careful weighing of many samples, or making numerous dilutions, which are subject to an error of sedimentation if suspensions are used. The second is rapid because it merely involves the operation of a shut-off device.

The type of deposition obtained with the two methods is different. Increasing the concentration, with spray time held constant, increases the quantity of material in the separate spray droplets without affecting the percentage of slide area covered. Increasing the spray time, with concentration held constant, increases the percentage area covered by increasing the size and number of spray droplets deposited on the slide. The question arose as to whether the two methods gave comparable results.

An experiment using the technique described below was made to ascertain the effect of these variables on fungicidal value. Suspensions of yellow cuprous oxide containing 0.2 per cent, 0.1 per cent and 0.05 per cent copper were made up. The 3 suspensions were sprayed on slides for 12, 6, and 3 seconds, respectively. Spores of *Macrosporium sarcinaeforme* Cav. were used. (Table 2.) When the data were plotted on logarithmic-probability paper (Fig. 4, A), according to Wilcoxon and McCallan (15), a straight line was formed, indicating that spore inhibition was a function of the log. dose of copper in micrograms per sq. cm. It was found also that equal copper depositions on the slide gave equal spore inhibition; *i.e.*, spores were equally inhibited by spraying X concentration for Y seconds as by spraying 2X concentration for 1/2Y seconds and *vice versa*.

This would indicate that the deposit of copper per unit area and not the percentage of area covered affects the fungicidal value. Stated otherwise this means that the amount of copper covered by the spore drop is the important factor, and not the amount in each spray droplet nor the type of deposit on the slide. It should be pointed out, however, that in

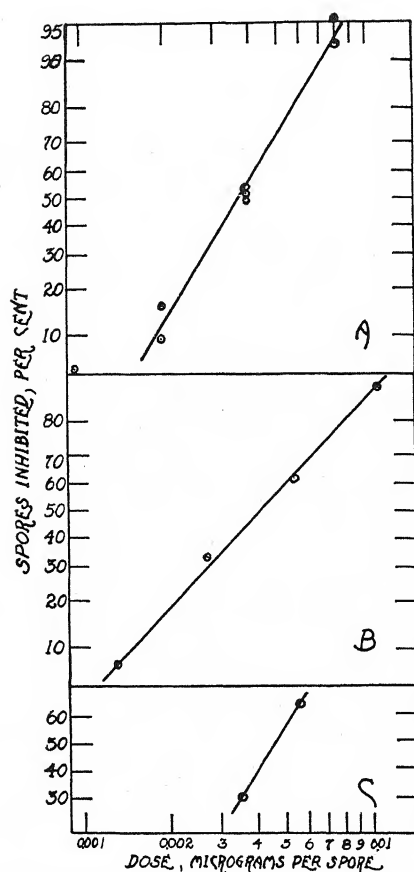


FIG. 4. Effect of dosage of toxicant per spore on inhibition of spore germination. A. Spore concentration and drop size constant, deposition variable. B. Deposition and drop size constant, spore concentration variable. C. Deposition and spore concentration constant, drop size variable.

TABLE 2.—Effect of varying both spraying time and concentration on fungicidal value of yellow cuprous oxide

Concentration of copper	Spraying time	Deposition of copper micrograms per sq. cm.	Inhibition
<i>Per cent</i>	<i>Seconds</i>		<i>Per cent</i>
0.05	12	3.84	52.1
	6	1.92	9.3
	3	0.96	5.6
0.10	12	7.68	95.0
	6	3.84	49.4
	3	1.92	16.5
0.20	12	15.36	100.0
	6	7.68	92.5
	3	3.84	53.2

all cases the spore drop covered the deposit from many spray droplets. Therefore, this generalization is good only insofar as the spray droplets were sufficiently close together so that the deposits from several droplets are covered by the spore drop.

This conclusion seems to be invalid, however, in the case of sulphur fungicides where the germination varies with the distance between the spray droplets. In other words, the inhibition of spores with copper appears to be a function of the quantity enclosed by the spore drop, but the inhibition of spores with sulphur appears to be a function of the percentage of slide area covered.

Since spraying time obviously affects the quantity of deposition, the best method of regulating spraying time is of interest. If a vertically sliding shutter is placed immediately before the target, the excess spray fluid tends to drip from the shutter into the spray stream and be blown onto the target giving unpredictable deposition. Placing it immediately in front of the nozzle introduces an additional error of lag because of the time required to set in motion the air of the spray stream. This lag might be important for points on the steep part of the curve. A pinch cock in the compressed air line eliminates the error from drip, but continues the error of lag. The pinch cock introduces another error of lag because time is required to lift the liquid from the level in the container to the nozzle. Since the errors of drip seem to exceed those of lag, the pinch cock is now used. In practice a stop watch is used to measure the spraying time, and 7 exposures are used to give 7 deposits.

THE FUNGUS

Often the fungi that have been utilized for biological assays have performed so erratically that it has been difficult to measure differences among protectant fungicides. Wilcoxon and McCallan (14) have overcome the matter of erratic spore germination by the addition of growth-promoting substances to the spore suspension. They found that very dilute orange juice was a satisfactory stimulant to spore germination. It seems a bit odd, however, to encourage the spore with orange juice on the one hand and to discourage it with a toxicant on the other. The results might appear to depend upon just how serious was the jolt in relation to the stimulant.

Macrosporium sarcinaeforme Cav. is suggested as an indicator fungus that requires no artificial stimulant. This fungus is pathogenic to *Trifolium pratense*. Its possibilities as a laboratory test fungus were discovered during a routine survey of the reaction of forage crop pathogens to fungicides (5). It has been used at various times since as an indicator fungus (3, 4, 6, 15).

Scant aerial mycelium is produced on oat agar at 20° C. The spores are large and black. Their hyaline germ tubes are easily distinguished from

spores and from hyphal fragments. Usually, 98–99 per cent of the spores germinate in distilled water at 25° C.⁷

This fungus has functioned well in the copper-fungicide studies and in the research now under way on organic fungicides. It does not react well to elemental sulphur. The writers would be pleased to provide single-spore cultures of this organism to anyone interested.

It is debatable whether more than one fungus should be used as an indicator. There is much evidence that many fungi respond similarly to different toxic chemicals (8, 9, 13). Different organisms, however, do not always react similarly to a series of compounds (2).

Resistance Level. Perhaps the most important variable encountered in using a fungus as a biological indicator is its resistance level. Resistance level may be defined as the response of a spore population to a standard fungicide. It should be emphasized that resistance level is not a response of the individual spore but of the population. Most of the day-to-day variations in laboratory tests that are not traceable to spraying error are traceable to oscillations in the resistance level of the spore population.

Age of Spores. McCallan and Wilcoxon (10) have shown that as spores age, their resistance level falls; *i.e.*, they become more easily inhibited. In practice spore age cannot be ascertained with certainty, so that the age of the transfer must be used as a criterion. In one test, using yellow cuprous oxide at 5.03 micrograms of copper per square centimeter, of sprayed surface the percentage inhibition for spores from 8-, 11-, 21-, 32-, and 43-day-old cultures of *Macrosporium sarcinaeforme* was 3.5, 18.5, 87.0, 100.0 and 100.0 per cent, respectively. Since spores of this fungus are produced rather slowly, 21 ± 4 -day-old cultures are generally used.

Inoculum Potential. Inoculum potential is just as important in testing fungicidal value as it has been shown to be in testing seed-protective value (7). In fungicide research inoculum potential refers to the amount of inoculum to be inhibited by the toxicant. The inoculum potential varies with the number and size of spores in the suspension and with the area covered by the spore drop. Inoculum potential is useful in calculating the dosage per spore. It has been shown (Table 2) that response varies directly with the logarithm of the toxicant dose when inoculum potential is kept constant. Stated otherwise, this means that the response varies with the log. dose per spore. In several other tests the toxicant dosage has been kept constant and the inoculum potential has been varied.

In a typical experiment several slides were sprayed with a red cuprous oxide suspension to give 6.20 micrograms of copper (toxicant) per square centimeter of sprayed surface. Seventeen-day-old cultures of *Macrosporium sarcinaeforme* were employed in preparing spore suspensions varying from 5,000 to 40,000 spores per cc. Since each spore drop spread to 7.5

⁷ When used at Long Ashton, England (6), however, the spores would not germinate in the distilled water available, and the reasons were not discovered. They germinated profusely in boiled tap water.

mm. in diameter, this exposed each spore to dosage varying from 0.01096 to 0.00137 micrograms of copper. The data plotted on logarithmic-probability paper (Fig. 4 B) gave a straight line, showing again that response varies with the log. dose per spore.

Another element in inoculum potential is size of spore drop. Obviously, as the area covered by the spore drop changes, the dose of toxicant per spore also changes, and in turn the response changes. If the unknown contains a spreader, the drop of spore suspension spreads more extensively, each spore is exposed to more toxicant, and the inhibition is higher than expected.

In a typical experiment, red cuprous oxide with and without sulfite lye (a weak spreader) was sprayed to give 5.03 micrograms of copper per square centimeter. The spore suspension contained 15,000 per cc. Without spreader the 0.05 cc. drop of spores spread to 0.524 sq. cm., thus exposing each spore to 0.0035 micrograms of copper, which inhibited 30.4 per cent of them. With spreader, the drop spread to 0.709 sq. cm., thus exposing each spore to 0.00542 micrograms of copper, which inhibited 64.2 per cent of them. In this case a 50 per cent increase in area of drop doubled the spore inhibition.

When these 2 points are placed on logarithmic-probability paper (Fig. 4 C) as before, they form a straight line with essentially the same slope as the data from the other experiments, showing again that response is a function of log. dose per spore.

From these experiments it follows that the important element in dosage studies is not concentration in the spray tank, not deposition per unit of sprayed area, but rather the quantity per spore. This quantity varies with the size of spore drop, spore concentration, and deposition per unit of sprayed area.

Sometimes it is necessary to test materials containing spreaders or to test detergents themselves. In that case it has been necessary to affix 15 mm. round cover glasses to the slide and spray these. Four drops of spores teased out to the edge of the cover glass give essentially the same distribution as one drop on cellulose nitrate. By teasing the spore suspension out to the edge, the spore distribution is equal whether the material has a spreader or not.

Size of spore is still another element in inoculum potential. It is a common impression that *Macrosporium sarcinaeforme* is less sensitive to copper than *Sclerotinia fructicola* (Wint.) Rehm. Each spore is much larger, however, and should be more difficult to inhibit. Conidia of *M. sarcinaeforme* average $28.01 \times 23.39 \mu$ in size (5). Those of *S. fructicola* average $28 \times 12 \mu$. Assuming that each conidium is an oblate spheroid the volume is $76867 \mu^3$ and $20106 \mu^3$, respectively. That is, each spore of *M. sarcinaeforme* contains 3.8 times as much material as that of *S. fructicola*. An attempt, therefore, was made to expose equal spore volumes of the two fungi to equal copper. Suspensions of 5,000 20-day-old spores of *M. sarcinaeforme* and $3.8 \times 5,000$ or 19,000 10-day-old spores of *S. fructicola* were

prepared. Drops of these were placed side by side on different slides sprayed with 3 lots of Coposil. The deposits to inhibit 50 per cent of the spores (LD50) for the 3 lots of Coposil and for the two lots of spores in micrograms per square centimeter were 2.8 and 3.3, 1.1 and 1.5, 3.5 and 3.3, respectively. These pairs of figures agree sufficiently well to indicate that the protoplasm within the two spores is equally sensitive, provided equal volumes are exposed to the toxic substance.

Methods of Handling the Fungus. Spores from 21 ± 4 -day transfers of *Macrosporium sarcinaeforme*, grown on oat agar at 20° C., were washed from the slants with conductivity water. The number of spores per cc. was determined (11) with a Levy haemocytometer with the Fuchs-Rosenthal ruling. Sampling the spore suspension had to be rapid to reduce errors of sedimentation. Enough water was then added to the suspension to adjust it to 5,000 spores per cu. cm.

Sprayed slides were lined up along the table and single drops of spores were placed on each in order until two drops were on each one. The drops were allowed to fall from a pipette held at a 45° angle^s and from a height of 1 cm.

The slides were placed immediately on glass racks in inverted moist chambers, with a water seal. After an incubation period of from 16 to 20 hours, the drops were examined. Originally 3 spray deposits were used for each material with 600 spores per exposure, making 1800 spores per treatment. Precision was gained by going to 5 exposures and 200 spores per exposure and finally to 7 exposures and 100 spores per exposure. This precision was gained despite a saving in the counting of 1100 spores per treatment.

Counting was facilitated by using a Breed eyepiece micrometer in the ocular of the microscope. The spores not germinated were recorded. Any spore with a tube long enough to have parallel sides was classed as germinated. No effort was made to measure the germ tubes, because the additional information gained hardly seemed to warrant the additional effort (15).

METHODS OF CALCULATING DATA

The problem of expressing fungicidal value is of some importance. The theoretical aspects of the subject have recently been discussed in some detail by Wilcoxon and McCallan (15).

The LD50 Value

Trevan (16) proposed a useful term, LD50, meaning lethal dose for 50 per cent, or the quantity of material required to kill or inhibit 50 per cent of the test organisms. The justification for using the LD50 value is that points near 50 per cent can be determined with the most precision and give the most information of any points on the curve.

^s The pipette is held at an angle, rather than vertically, in order to reduce the error of settling of the large spores.

It is well, however, to obtain sufficient data on any unknown to draw the complete curve of fungicidal value. That is, enough deposits should be used to give data over the whole range 0 to 100 per cent inhibition. From these data fungicidal value curves can be plotted on logarithmic-probability paper (15), the abscissa being deposition of toxicant in micrograms per square centimeter, and the ordinate being the percentage of spores inhibited. This curve will serve to determine LD50 or any other LD point by interpolation.

STANDARD FUNGICIDE

A non-sprayed slide was carried in every test as a check on the viability of the spores. Since all the factors governing resistance level and all the variables in spraying have not yet been brought under control, a day-to-day experimental error remains. The magnitude of this error must be known if materials tested on different days are to be compared accurately. A standard fungicide was designed to measure this error on the assumption that there are fewer errors in preparing the standard than in the rest of the technique. It is assumed that a variation in spore response to the standard will be reflected equally in the response to the unknown under test.

The choice of a standard fungicide has occasioned considerable deliberation. The specifications were simple. The material should be well known, should be easily obtainable, should have known composition, and should possess a median spore-inhibiting capacity, *i.e.*, be neither too potent nor too weak. One of the so-called insoluble copper compounds in the middle range would suffice although such selection would involve problems of commercial connotations.

In considering a standard, Bordeaux automatically comes to mind. Apparently, its chief advantages are that it is easily prepared, is famous, and that it requires no commercial preparation. On the other hand, Bordeaux is an uncertain complex; it has almost the highest spore-inhibiting power of any copper material now in use and hence does not admit of both plus and minus variation as a standard should. The slope of its fungicidal value curve is very steep, which is advantageous in a standard because differences in resistance level of the fungus, which are reflected in spore inhibition, are minimized when read as deposition. Since the standard is designed to convert resistance level to deposit, a steep slope is conducive to best results.

Bordeaux, therefore, was chosen for a standard. The fact that its composition is uncertain probably is unimportant as long as it can be reproduced at will. The composition of Bordeaux varies with the method of preparation, temperature, and composition of ingredients. These were all standardized. Stock solutions, instead of solids, were used to insure rapid and uniform reactions. Temperature was held between 20° and 25° C., and reagent quality ingredients, free of carbonates, were employed.

Stock Copper Sulphate. A 0.3928 per cent solution of reagent quality

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0% Cu) was prepared. It was kept in a siphon bottle with attached burette.

Stock Lime Water. A saturated lime-water solution was prepared by suspending an excess of reagent quality hydrated lime in boiled distilled water. It was agitated thoroughly to obtain saturation and allowed to settle. It was kept in a siphon bottle protected from CO_2 with soda-lime tower.

Standard Bordeaux. The volume ratio of stock copper sulphate and lime water was kept at 1 to 12 (mol. ratio 1 Cu to 1.65 Ca). The stock copper sulphate was added to the stock lime water while stirring. The quantities in cc. of stock copper sulphate to be added to stock lime water and diluted to obtain varying percentages of copper appear in table 3.

TABLE 3.—Quantities of stock materials to give standard Bordeaux

Final volume in cc.						Copper
250		500		1000		
Copper	Lime	Copper	Lime	Copper	Lime	
50	60	100	120	200	240	<i>Per cent</i>
25	30	50	60	100	120	0.02
.....	25	30	50	60	0.01
						0.005

In a practical determination of resistance level, the standard fungicide was applied by spraying, as already described. Since resistance level could not be predicted, it was necessary in all tests to apply 3 copper depositions above, at, and below the normal resistance level. A typical experiment may be cited. The technique was standardized as follows:

Spraying distance	50 cm.
Sprayed surface	Cellulose nitrate on glass
Concentration of copper in Bordeaux	0.005 per cent
Spray fluid deposited by atomizer	0.64 mg. cm^2/sec .
Copper deposited by atomizer	0.032 micrograms cm^2/sec .
Fungus	<i>Macrosporium sarcinaeforme</i>
Agar	Oatmeal
Age of transfer	21 days
Temperature of growth	20° C.
Temperature of germination	20° C.
Spore concentration	5,000/cc. in distilled water
Diameter of spore drop (0.05 cc.)	7.5 mm.
No. of spores	565/ cm^2

Three exposures of 3, 5.5, and 8 seconds gave deposits of 0.096, 0.176 and 0.256 micrograms of copper per sq. cm. of sprayed surface. In the experiment the percentage of spore inhibition was 8.0, 35.0, and 57.0 per cent, respectively. Interpolation on the curve, plotted on logarithmic-probability paper, showed that the LD50 or resistance level for the test in question was at 0.221 micrograms of copper per square centimeter, or 0.00039 micrograms per spore.

Bordeaux Coefficient

The variation in the resistance level of the spore population was reflected, of course, in the unknown material, as well as in the standard. Having arrived at a measure of resistance level, it was possible to compare tests on different days. This was done by calculating a Bordeaux coefficient for the unknown that was under test.

Bordeaux Coefficient Where Toxicant is Known. It was first necessary to determine for the unknown the LD50 point based on the toxicant (copper, lead sulphur, etc.). This was done by experiment. The Bordeaux coefficient was then calculated for each test according to the following formula:

$$\text{Bordeaux coefficient} = \frac{\text{LD50 deposition of copper in Bordeaux}}{\text{LD50 deposition of toxicant in fungicide } \bar{X}}$$

As expected, the variation in Bordeaux coefficient among tests was less than the variation in LD50 values. Ten tests have been made with a certain stock of red cuprous oxide. The coefficient of variation for the LD50 values was 45.4, but it dropped to 23.9 for the Bordeaux coefficient. This means that the daily variability in results could be cut in half by making use of a Bordeaux coefficient.

Bordeaux coefficient, however, does not provide enough information, because the fungicidal value curves of materials may vary in slope. Two materials may have equal Bordeaux coefficients, that is, be equally fungicidal at LD50 but may have different slopes. If so, the one with the steep slope will reach LD95 or LD100 with a smaller deposit than one with a flat slope. That is, it will be the more potent fungicide for complete fungus control.

Bordeaux Coefficient Where Toxicant is Uncertain. At present many materials other than those containing metallic toxicants are under test. In most of these the toxicant is unknown or uncertain. It is hardly logical in these cases to express the Bordeaux coefficient on the basis of the metallic copper in Bordeaux. Accordingly, it is suggested that the ratio be expressed on the basis of total solids. The standard Bordeaux just discussed contains 0.0598 per cent total solids.

DISCUSSION

Beginning in 1926, the writers have been interested in fungicides and their performance. As with other phases of agricultural research, the field work was impeded by uncontrolled variables. Upon analysis it became apparent that accelerated tests were needed for plant injury, resistance to weathering, and fungicidal value, *i.e.*, spore-inhibiting power. Special studies on these problems have been made since 1936. Little progress can be reported on plant injury. A technique for measuring and evaluating tenacity is being published elsewhere (3).

This paper describes the design of a precision sprayer and it discusses the measurement of resistance level of the spore population and the calcula-

tion of a Bordeaux coefficient to reduce the effect of changes in resistance level from day to day.

The professional practice of speaking in terms of concentration (*i.e.*, 4-4-50 Bordeaux) probably has delayed the design of a precision sprayer. It is not 4-4-50 Bordeaux that controls potato blight. It is the 4.06 lb. of copper applied to the foliage on an acre of potatoes when the rate of application is 200 gal. It is not enough to discuss the *concentration* of copper in a spray suspension. That is only one of the elements in *deposition* of copper on the sprayed surface. A precision sprayer is required to convert a precise concentration to a precise deposition.

A precision sprayer is not difficult to design when the governing factors are kept in mind: (1) a known spraying distance, (2) a known spraying time, (3) a constant air pressure, (4) a constant height of lift, (5) protection of air stream against eddying, (6) control of dew point of air that is sprayed through, (7) a spray fluid and a sprayed surface with a known, preferably a constant, surface tension.

Having obtained a precise deposit, its fungicidal value must be measured with precision. The fungus must be carefully handled (11) and the inoculum potential, *i.e.*, population density, must be held under strict control, because it has been found that the response is a function of the logarithm of the dose per spore.

There are 3 sources of error in maintaining this relation constant: (1) deposition of toxicant per unit area, (2) number of spores per cc. of initial suspension, and (3) area covered by each drop of spore suspension. The last point indicates the necessity of a uniform surface tension of spray fluid and sprayed surface. It indicates also that care must be taken in testing spreader-containing materials, because the spore drop will cover a larger area than normal and give a higher fungicidal value than the material actually possesses.

McCallan and Wilcoxon (10) have pointed out the elements of precision in handling the fungus: (1) known age, (2) constant spore concentration, (3) medium, (4) clean glassware, and (5) constant temperature.

It is only necessary to add that in comparing the resistance levels of different spore species, it is well to consider their relative sizes. It is also imperative that the spore suspension droplet spread over a constant area of sprayed surface or otherwise the spores will be exposed to more or less than the normal amount of protectant.

Finally, there are the problems connected with presenting the data. The concept of Bordeaux coefficient has been developed during the past 2 years as an aid to precision in this connection. At first an LD50 Bordeaux was devised to measure the resistance level of the spores under test. It has been practically impossible to keep the resistance level of the spores constant, as it has not yet been feasible to obtain a crop of spores every day of precisely the same age or of precisely the same concentration. LD50 Bordeaux shows the fluctuation in this level. If the resistance level falls,

a smaller amount of copper per square centimeter is required to inhibit 50 per cent of the spores. If it rises, a larger amount is required. Likewise, the deposition of the unknown to give 50 per cent inhibition also fluctuates with the resistance level. Since the standard and the unknown fluctuate together, the Bordeaux coefficient for the unknown shows less variability than its LD50 deposition.

It should be pointed out, however, that Bordeaux coefficient involves a shifting base line which must be used with discretion. It is true that Bordeaux coefficient is less variable than LD50 and is, therefore, very useful. On the other hand the coefficient for materials weaker than Bordeaux falls as resistance level rises and rises as resistance level falls. The coefficient for materials stronger than Bordeaux follows the reverse procedure. Suppose that 1.2 micrograms of copper, in Bordeaux are required per square inch of sprayed surface for LD50 and that 4.8 micrograms of copper in some other material are required. That gives a Bordeaux coefficient of 0.25. If resistance level rises 10 per cent, 1.32 micrograms of copper in Bordeaux are required and 5.28 in the unknown. The Bordeaux coefficient then falls to 0.242. The usefulness of the statistic lies in the fact that a 10 per cent change in the resistance level is reflected in only 0.3 per cent change in the coefficient. Nevertheless, it must be recognized as an aid for reducing variance, not as an absolute figure.

SUMMARY

In response to a need in the fungicide laboratory, alterations in procedure and equipment have been made for testing and determining the fungicidal value, *i.e.*, the spore-killing power of fungicides in the laboratory.

A precision sprayer is required in order to convert precise concentrations into precise deposits. The nozzle is a deVilbiss atomizer No. 15. The target is a 1 in. \times 3 in. glass microscope slide, coated with cellulose nitrate. These were fixed at opposite ends of a cylinder 4 in. wide and 30 in. long. By thus enclosing the spray stream it was not deflected by stray air currents.

It was found that the spray tended to evaporate and lose its load of toxicant at low air humidities. Accordingly the cylinder was placed inside a hood, where dew point could be controlled by varying the humidity. The target end projected through the side of the hood to facilitate changing slides and to prevent back pressure.

The deposit of spray fluid on the slide per unit of time was regulated by changing the size of the hole in the atomizer end of the tube.

The distance between the target and the atomizer and between the atomizer and level of spray in the container had to be maintained constant, because deposition varied inversely with these distances.

Since it was found that response varied with the logarithm of the dose per spore, inoculum potential had to be controlled.

Spore concentration was, therefore, held constant at 5,000 per cc. by

using a haemocytometer. The same pipette was used to give constant drop volume. Drops were allowed to fall from the same height to assure constant spread. The slide was coated with cellulose nitrate to obviate variations in spreading properties of glass. When a sample contained a spreader, a correction had to be made in the size of the drop of spore suspension.

The fungus used was *Macrosporium sarcinaeforme* which sporulates readily on oat agar, with a minimum of aerial hyphae, if grown at 20° C. Spores are large and black, with hyaline tubes easily distinguished from the black hyphal fragments.

It was found that a Breed eyepiece micrometer facilitated counting.

The dosage per unit area of sprayed surface was varied by altering the spray exposure. Seven spray exposures were made to explore the range of toxicity. One hundred spores, 50 in each of 2 drops, were counted for each spray exposure.

The preparation of a standard LD50 Bordeaux is described.

A Bordeaux coefficient is calculated to measure the relative fungicidal value of the material under test. It is the quotient obtained by dividing the quantity of copper in Bordeaux necessary to give LD50 by the quantity of copper in the unknown necessary to give LD50. Bordeaux coefficient gives a correction for oscillations in resistance level of the spores, and makes possible a comparison of materials tested on different days.

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EFFECTS OF H-ION AND AL-ION CONCENTRATIONS ON DAMPING-OFF OF CONIFERS AND CERTAIN CAUSATIVE FUNGI¹

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INTRODUCTION

The disease of conifer seedlings known as damping-off is a symptomatic category of diseases caused by soil-inhabiting fungi. The diseases are characterized by the rapid decay of portions of the root, hypocotyl, and cotyledons. These diseases, as a group, are sometimes further differentiated on the basis of the time of infection, which may be any time from the inception of germination until the seedlings are several weeks old.

Since the most prevalent types of damping-off are caused by soil-inhabiting fungi attacking the root or lower portion of the hypocotyl, the most widely used control measures have been soil treatments. Among these, certain acid treatments have proved successful at a number of forest nurseries in the United States. The sulphuric acid treatment was introduced by Spaulding (23) in 1908. Extensive field investigations (8, 9) since then have demonstrated the effectiveness of sulphuric acid against damping-off of conifers, though in most places, it gives only partial control; at occasional nurseries it is practically worthless. Kay (12) reported sulphuric acid effective against damping-off in nursery seedbeds in Alberta, Canada. On the other hand, Doran (5) used inorganic acids against tobacco root rot caused by *Thielavia basicola* (B. and Br.) Zopf and found that sulphuric acid and nitric acid were too toxic for further trials. He also observed that aluminum sulphate reduced the severity of the disease. Hansen *et al.* (7) obtained control of damping-off of conifer seedlings with hydrochloric acid at a forest nursery in northern Minnesota.

Hartley (10), in 1928, and Wiant (26), in 1929, reported on the efficacy of aluminum sulphate as a damping-off treatment. The results from their experiments indicated that it was equal, or even superior in some cases, to

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sulphuric acid for controlling damping-off at several forest nurseries. At Monument, Colorado, aluminum sulphate was particularly effective against a root rot that caused heavy losses of first-year and dwarfing of older seedlings of Douglas fir (*Pseudotsuga taxifolia* Britt.).

The apparent effectiveness of soil treatments with either inorganic acids or aluminum sulphate against damping-off has raised several questions in regard to the relative effects of the H-ion and the Al-ion concentration on the development of damping-off. It was for the purpose of obtaining further information on these questions that the investigations here reported were undertaken. The principal objectives of the study were: (1) the effect of pH on damping-off in the absence of the Al-ion, (2) the effect of pH on the growth of damping-off fungi, and (3) the effect of Al-ion concentration on damping-off when pH is held constant.

HOSTS AND FUNGI

The selection of hosts and damping-off fungi was based on results from extensive experiments in a nursery at Monument, Colorado, conducted by W. H. Schrader of the U. S. Forest Service in collaboration with the Bureau of Plant Industry. In this nursery sulphuric acid and aluminum sulphate successfully controlled damping-off of ponderosa pine (*Pinus ponderosa* Laws.) and Douglas fir, as well as the subsequent stunting of the surviving Douglas fir. The isolations, made by G. G. Hahn and the writer, consistently yielded a *Pythium*, which was considered the principal parasite. The *Pythium* isolates from Monument were tentatively identified by Charles Drechsler as *P. ultimum* Trow. The above-mentioned hosts were used in all the experiments, and Schrader collected the seed of both species from the same locality in the Pike National Forest. The sources of the fungi used in the experiments are given in table 1.

TABLE 1.—*Sources of the fungi used in the experiments on the relation of the H-ion and Al-ion to damping-off Douglas fir and ponderosa pine seedlings in liquid and quartz-sand cultures*

Isolate No.	Species	Host and disease	Year of isolation	Locality
1140	<i>Pythium</i> sp.	<i>Pinus aristata</i> Engl. (root rot)	1927	Monument, Colo.
1145	" "	Douglas fir (damping-off)	1925	" "
1151	" "	Douglas fir (root rot)	1927	" "
1299	" "	" " " "	1928	" "
1310	<i>Rhizoctonia solani</i> Kühn	Red pine (damping-off)	1930	College Park, Md.
1320	" "	" " " "	1930	" " "
1341	" "	Douglas fir	Minnesota
42	<i>Pythium</i> sp.	" " (damping-off)	1930	Philadelphia, Pa.
82 ^a	" "	Sugar beet (damping-off)	Colorado
86 ^a	" "	" " " "	Colorado

^a Cultures furnished by F. G. Larmer.

METHODS

The seeds were surface-sterilized for 2 minutes in an aqueous solution of 1:1000 mercuric chloride, rinsed in 5 changes of sterilized distilled water,

and then germinated on sterilized peat moss that had a pH of about 3.5. The seedlings were transplanted to the liquid and quartz-sand cultures when the radicles were from 5 to 10 mm. long. The liquid cultures were run in 1-l. glass beakers, and the seedlings were arranged in a double support; the upper part was a piece of paraffin, shaped like a Maltese cross, with 25 holes along the edges for the seedlings; the lower part was a circular piece of paraffin-impregnated bobbinet, which was immersed 5 mm. below the liquid surface and was used to support the inoculum. Both platforms were attached to an adjustable glass rod. It was necessary to renew the solutions every 2nd, 3rd, or 4th day in order to keep the reaction changes less than 0.4 of a pH. The quartz-sand cultures were run in $\frac{1}{2}$ - and 1-gal. glazed crocks, with sand that had been washed first with hot and cold tap water and then with distilled water. The solutions were renewed often enough, usually every other day, to keep the pH of the drainage solution within 0.3 pH of the desired points. Drainage was obtained by a suction system. At each renewal the old solution was washed out with distilled water, and the sand surface was then flooded twice with the fresh solution at 0.5 of a pH below the desired point, since the sand usually raised the pH of the drainage water by 0.5 of a pH.

The inoculum used in the damping-off experiments consisted of 3-mm. cubes cut from the margins of cultures of the fungi grown in Petri plates on commercially prepared corn-meal agar with a pH of about 6.0. The inoculations in the liquid and the quartz-sand cultures were made by placing the inoculum approximately 5 mm. below the surface of the medium, and at a distance from the seedlings varying from 2 to 5 mm. Thus, both the parasite and the inoculated part of the host were subjected as far as possible to the effects of the H-ion and the Al-ion concentrations of the media.

The seedlings were recorded as damped off when they toppled over from infection. The amount of damping-off in each culture was calculated as the percentage of the total number of inoculated seedlings. The liquid and the quartz-sand experiments were run until damping-off ceased.

The selection of a nutrient solution was difficult because, when the work was done in 1932, the literature revealed very few that had been used for conifer seedlings. Weis (24) observed satisfactory growth of certain conifers in Hansteen-Cranner's ammonium sulphate solution. The work of Herbert (11) on the growth of black spruce in nutrient media yielded a few suggestions. The chief objection to Skeen's (22) weak solution, as with many others, was the precipitation that occurred above pH 7.0. Further complications were encountered when aluminum was added, for, as Line (13) stated, aluminum hydroxides precipitate above pH 4.0 and aluminum phosphates between pH 3.0 and 4.0.

The greatest possibility for a solution that would be stable from pH 2.5 to 8.5, when Fe- and Al-ions are added, was offered by the use of the glycerinated phosphates. Sodium glycerophosphate [$\text{Na}_2\text{C}_3\text{H}_5(\text{OH})_2\text{PO}_4$] was successfully used by Mellon *et al.* (17) for the culture of bacteria and

fungi. They found that the ionization-constant for glycerophosphoric acid was close enough to that of phosphoric acid to permit its use as a buffer. In another paper (2) they reported: "Glycerophosphates, sucrose, and mannite phosphates and others are sources of carbohydrate food as well as of phosphorus." They also stated that the calcium, magnesium, and other salts of glycerophosphoric acid are soluble. Glycerophosphate was used by Allen (1) to culture *Azotobacter*. He stated also that the phosphates were available to some extent in alkaline solutions. Ferric glycerophosphate has been used as an iron source (14, 15, 16), especially in neutral and alkaline solutions. The data on the composition of the nutrient solutions used in the experiments are given in table 2.

TABLE 2.—Composition of the nutrient solutions used in the experiments on the relation of the H-ion and Al-ion to damping-off and growth of Douglas fir and ponderosa pine seedlings in liquid and quartz-sand cultures

Components	Molar concentration per liter		
	Skeen's weak solution minus aluminum	Glycerophosphate B	Glycerophosphate C
NH ₄ NO ₃003
Ca(NO ₃) ₂008	.007	.002
CaCl ₂003
H ₃ PO ₄01
Na ₂ C ₆ H ₅ (OH) ₂ PO ₄004	.003
KCl001	.001
MgSO ₄004	.002	.0015
Ferric citrate	1 ml. 1%	1 ml. 1%	1 ml. 1%

The reaction of Skeen's solution was adjusted with 0.1N KOH, and the glycerophosphate solutions were adjusted with 0.1N HCl or 0.1N NaOH. The pH determinations were made electrometrically with quinhydrone.

EXPERIMENTAL RESULTS

The Effect of pH on the Growth of Damping-off Fungi in Liquid Cultures

For the study of the effect of pH on growth of damping-off fungi, an apparatus was devised whereby the nutrient solution could be renewed frequently enough to hold the pH constant. The culture flask was similar to that used by Sideris (21), except that the inlet was changed to a funnel, made from an 18-mm. test tube and plugged with cotton. A glass rod was placed in each flask to furnish a place of attachment for the mycelium, which aided the withdrawal of solutions. Glycerophosphate solution B was used in the cultures. The pH of the solutions in the series varied by one unit from 2.5 to 8.5. The small amount of carbon contained in glycerophosphate was adequate for the growth of *Pythium* and *Rhizoctonia*. All the cultures were grown in darkness in a constant-temperature room at approxi-

TABLE 3.—Effect of pH on the growth of *Pythium* and *Rhizoctonia* isolates in glycerophosphate solution B

Experiments and isolates	Growth at indicated pH						
	2.5	3.5	4.5	5.5	6.5	7.5	8.5
Solution B: Experiment 1— <i>Pythium</i> 1145 (2 series)	None	None	Much thin mycelium; oospores, chlamydospores few	Much dense mycelium; oospores, chlamydospores abundant	Much dense mycelium; oospores, chlamydospores abundant	Sparse and thin mycelium; oospores, chlamydospores less abundant	None
Experiment 2— <i>Pythium</i> 1299 (2 series)	"	"	"	"	"	"	Trace
Solution B plus 1% sucrose: Experiment 3— <i>Pythium</i> 1299 (2 series)	"	"	Much thin mycelium; no spores	Much dense mycelium; no spores	Much dense mycelium; no spores	Sparse and thin mycelium; no spores	None
<i>Pythium</i> 82 (1 series)	"	"	"	"	"	"	"
<i>Pythium</i> 86 (1 series)	"	"	"	"	"	"	"
Solution B: Experiment 4— <i>Rhizoctonia</i> 1341 (2 series)	"	Sparse, and thin mycelium; no sclerotia	Much pale, brown mycelium; no sclerotia	Much dense, dark-brown mycelium; small sclerotia	Much dense, dark-brown mycelium; many sclerotia	Much thin, pale-yellow mycelium; no sclerotia	Sparse, thin, and pale-yellow mycelium; no sclerotia
Experiment 5— <i>Rhizoctonia</i> 1320 (1 series)	"	"	"	"	"	"	None

mately 20° C. The solutions had to be renewed every second day in order to keep the pH constant at the various points.

The results from the experiments on the effect of pH on the growth of damping-off fungi are tabulated in table 3. At pH 2.5 and 3.5, the *Pythium* isolates failed to produce visible growth. At pH 8.5, a trace of visible growth occurred only in the series in experiment 2. The largest quantity of growth appeared to occur at pH 5.5 and 6.5. The effect of pH on the growth of *Pythium* has been observed by other investigators. Flor (6) found that pH 4.6 was the lower limit for a strain of *Pythium* from sugar cane (probably not *P. ultimum*), and good growth occurred between pH 5.3 and 9.2. Roth (19) observed that a *Pythium*, to which he refers as *P. debaryanum* Hess., did not grow below pH 3.1, and the optimum was about pH 6.4. Schaffnit and Meyer-Herman (20) observed that the growth of *P. debaryanum* in the soil cultures was poor at pH 4.8 and best at pH 6.6, 7.1 and 7.4. On the other hand, White (25) reported that a *Pythium* from *Rhododendron* had a wider range of adaptability to acidity than the host.

The sporulation of *Pythium*, especially its oospore production, was favored in general by the same acidities that favored mycelial development. Chlamydospore and oospore production definitely increased from pH 4.5 to 6.5, where it reached a maximum and then decreased at pH 7.5. The lack of spores in experiment 3 probably was due to the addition of sucrose; *Pythium* isolates from damped-off conifers also failed to produce oospores in agar containing dextrose.

The pH of the media influences sporulation in other genera also. For example, Cooper and Porter (4) observed that a *Phytophthora* isolate, which caused peony blight, produced numerous oospores at pH 6.6 and 8.4, while at 5.3 there were comparatively few oospores and conidia.

Rhizoctonia, apparently was less affected by low pH than *Pythium*, since a small amount of growth occurred at pH 3.5. The largest amount of growth occurred at pH 5.5 and 6.5. Schaffnit and Meyer-Herman (20) observed that *R. solani*, when grown in soil cultures, formed a loose aerial mycelium at pH 4.3, and between 6.2 and 7.0 it formed a mycelium that grew more deeply in the soil. Roth (19) observed that *Corticium vagum* produced a small amount of growth at pH 3.2, but failed to grow at 2.9 and the optimum was at 6.5. Perhaps *Rhizoctonia* might still cause damping-off under the conditions of low soil pH by growing aerially over the surface. Monteith and Dahl (18) reported that *Rhizoctonia* was apparently able to grow over a wide range of acidity and alkalinity.

The results indicated that the growth of *Pythium* was more restricted by low pH than that of *Rhizoctonia*. Therefore, it ought to be somewhat easier to control *Pythium* than *Rhizoctonia* by an acidification treatment. The differences in parasitism, which developed at the various pH values in the liquid and the quartz-sand cultures, were probably somewhat dependent on the direct effect of the reaction of the media on the growth of the parasite.

The Effect of pH on Damping-off of Douglas Fir and Ponderosa Pine Seedlings in Liquid Cultures

The results from the experiments on the relation of pH to damping-off of Douglas fir and ponderosa pine are shown in figure 1. The experiments were run at different times. Douglas fir was used in 7 inoculated series and ponderosa pine in one series. Series 3, 6, 8, 10, and 12, for which no damping-off is shown in the graphs, were noninoculated Douglas fir cultures. In series 1 to 6, inclusive, the seedlings were grown in Skeen's weak solution; in series 7 to 13, inclusive, they were grown in glycerophosphate solution B. The reactions of the cultures were adjusted separately, and the pH varied by one unit from 2.5 to 7.5. The inoculations were made immediately prior to the seed-coat-shedding stage. The number of inoculated seedlings in the cultures varied as follows: 6 cultures with 12 to 19, 12 cultures with 20 to 24, and 29 cultures with 25 seedlings, though each culture was started with 25 seedlings. The variation in the number of seedlings was due to the unexplained failure of the roots of some seedlings to develop after they were transplanted.

Damping-off for *Pythium* and *Rhizoctonia* on both hosts was 0 in all the series run at pH 2.5. For *Pythium* on both hosts at pH 3.5, damping-off was 0 in 2 series, 24 per cent or lower in 4 series, and as high as 42 per cent in 1 series. There occurred a definite increase in the amount of damping-off for *Pythium* on both hosts from pH 2.5 or 3.5 to maxima at pH 5.5 or 6.5, then decreased in all but one of the series at pH 6.5 or 7.5. In the single test made with *Rhizoctonia*, the result in general was the same as for *Pythium*. There were 5 noninoculated series of Douglas fir, and damping-off was 0 in all; there were no noninoculated series with ponderosa pine. The decreased damping-off at pH 7.5 is in agreement with the decreased growth of the fungi at pH 7.5 reported in table 3, but is not in accord with field experience, in which damping-off is generally more severe at pH 7.0 or 8.0 than at lower pH. There was distinctly more damping-off in the *Pythium* cultures grown in Skeen's solution than in those grown in the glycerophosphate solution. There is $2\frac{1}{2}$ times as much phosphate in Skeen's solution as in solution B or C, which may have had an effect on the amount of damping-off in these solutions. This apparent effect of the difference in composition of the two solutions on damping-off needs confirmation, since the series were run at different times and with different isolates. There was no indication that the difference in the solutions affected the relation of damping-off to pH.

The Effect of pH on the Growth of Douglas Fir Seedlings in Liquid Cultures

When the damping-off experiments just described were terminated the Douglas fir seedlings from the noninoculated series were measured for total length in order to determine the effect of pH on growth. The total length was measured from the base of the cotyledon whorl to the tip of

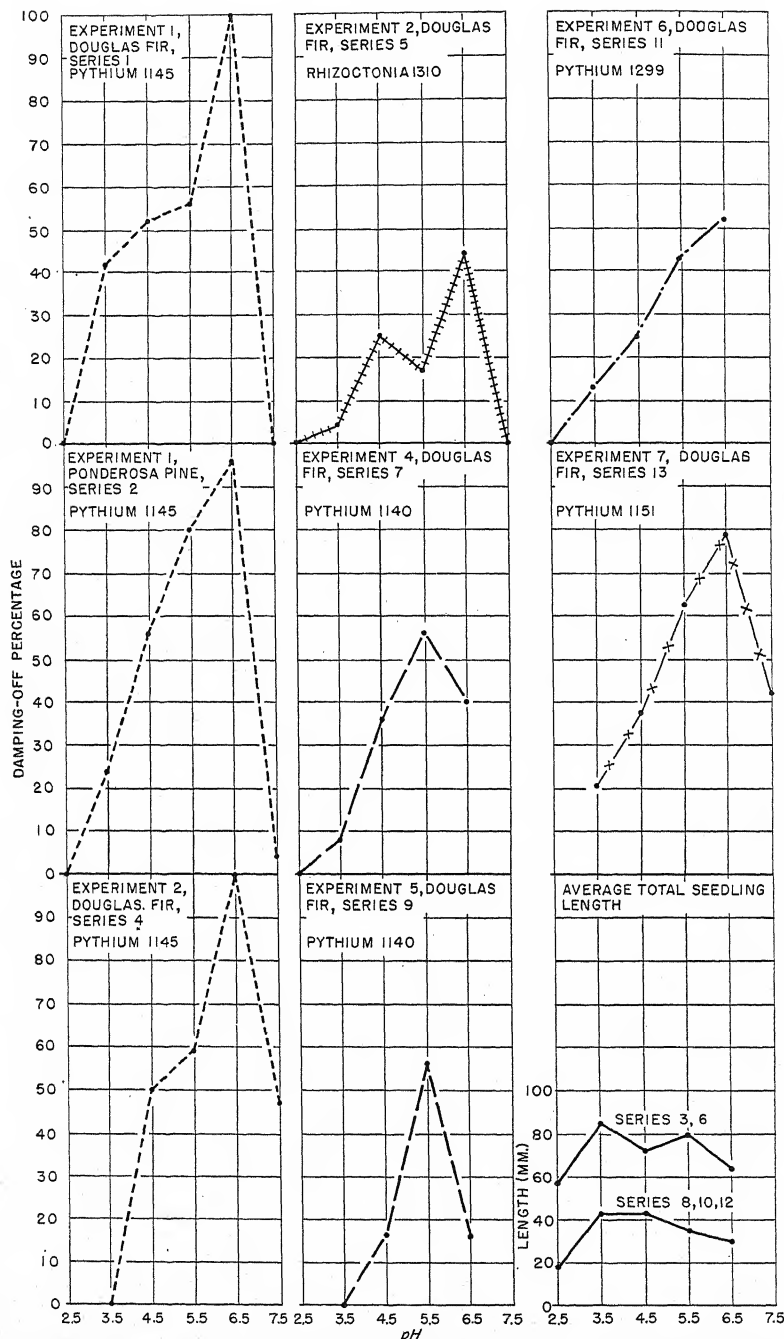


FIG. 1. Effect of pH on damping-off in liquid cultures. The 5 noninoculated series with Douglas fir, in all of which the damping-off losses were 0, are not shown; the average total length of seedlings in these is shown in lower right corner. Series 1-6 in Skeen's weak solution; 7-13 in glycerophosphate B.

the taproot. It is practically impossible to obtain a reliable measure of the root of a seedling, since the demarcation between root and hypocotyl is very indistinct; differences in total length, however, were largely due to root differences, since hypocotyl length is less variable. The results are tabulated in table 4 and summarized in the lower right corner of figure 1.

TABLE 4.—*The growth of Douglas fir seedlings in the noninoculated series in the liquid cultures*

Series and solutions	Average number of seedlings	Average total length in mm. at indicated pH					
		2.5	3.5	4.5	5.5	6.5	7.5
Skeen's weak-solution:							
3	24	73	94	80	94	73	66
6	20	40	75	62	63	53
Glycerophosphate B:							
8	22	26	58	52	36	35
10	25	9	30	28	29	22
12	22	18	40	46	41	30

The Douglas fir seedlings in the noninoculated liquid cultures, as shown in table 4, made fair growth in most of the series at pH 2.5. A definite increase in growth occurred from pH 2.5 to 3.5 in all the series. In 4 of the 5 series the growth at pH 3.5 either equalled or exceeded the growth that occurred at pH 5.5 and 6.5. Although the largest amount of growth appeared to occur at pH 3.5 and 5.5, the results were too variable to establish definite maxima. The series cannot be critically compared, since they were run at different times. However, it is interesting to note that the best growth, as well as the heaviest damping-off, occurred in the experiments in which Skeen's solution, with its higher but inorganic phosphorus content, was used.

Effect of pH on Damping-off of Douglas Fir and Ponderosa Pine Seedlings in Quartz-sand Cultures

The results from the experiments on the relation of pH to damping-off of Douglas fir and ponderosa pine in sand cultures are shown in figure 2. Series 1 to 10, inclusive, were grown in glycerophosphate B, and those from 11 to 17, inclusive, were grown in glycerophosphate C. The seedlings were inoculated at the seedcoat-shedding stage. The number of inoculated seedlings in the cultures varied from 14 to 28, though each had the same initial number of germinated seed. This variation was due to the unexplained failure of the roots of some of the seedlings to develop after they were transplanted. The reaction in each series varied by one unit from pH 2.5 to 8.5.

In the series run at pH 2.5, the amount of damping-off for both hosts in the *Pythium* cultures was 0 in two series, 6 per cent or less in two series, and as high as 29 per cent in one series. The amount of damping-off for both hosts in the *Rhizoctonia* cultures at pH 2.5 was likewise very

small. The cultures at pH 2.5 were discontinued ultimately because of poor root growth.

At pH 3.5 the amount of damping-off for both hosts in the *Pythium* cultures was low in 5 series with 10 to 20 per cent, fairly high in 3 series with 27 to 42 per cent, and exceedingly high in 1 series with 94 per cent. In the *Rhizoctonia* cultures it was low in 1 series with 11 per cent, and high in the other 2 series with 50 and 88 per cent, respectively.

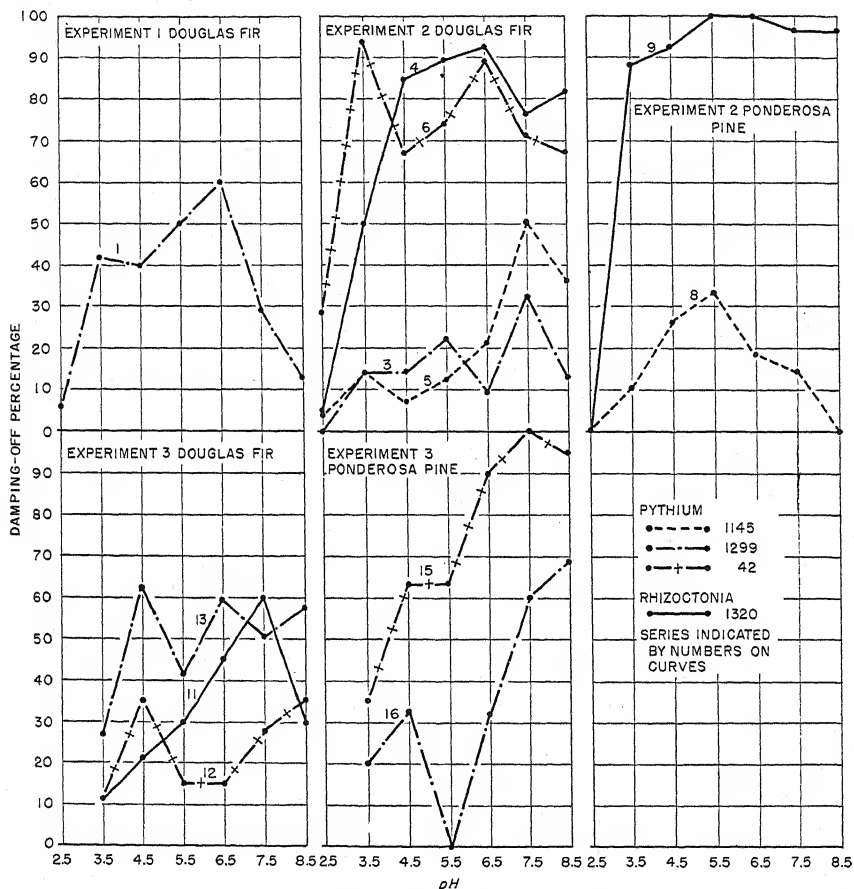


FIG. 2. Effect of pH on damping-off in quartz-sand cultures. Three noninoculated series with Douglas fir and 2 with ponderosa pine, in all of which the damping-off losses were 0, are not shown. Series 1-10 in glycerophosphate B; 11-17 in glycerophosphate C.

In the sand series the damping-off caused by *Pythium* was not so definitely related to pH as it appeared to be in the liquid-culture experiments. As in the liquid cultures, the damping-off in the sand series was always less at pH 2.5 than at higher values in the 5 series in which that pH was tested, but some disease did occur at this low pH in 3 series. In 3 of the 9 series there was no definite increase in damping-off with pH increase after 3.5 was reached, and in most of the series the upward trend of

damping-off with pH was less consistent than in the liquid cultures. The downward trend of damping-off with the highest pH values, seen so definitely in the liquid cultures, occurred in only part of the sand cultures. In the series grown in solution C, at pH 8.5, damping-off averaged as high as or higher than at the lower pH values, and thus agreed with nursery experience rather than with liquid-culture results. Because there was no indication of difference in reaction of the 2 host species, the results for all series have been combined in figure 3; these summary graphs indicate rather clearly that there is some relation of the disease to pH, and make it appear that the damping-off activity of the eastern *Pythium* No. 42 is affected by acidity in about the same way as the *Pythium* isolates that come from a western habitat of higher pH.

There were 3 noninoculated series with Douglas fir, and 2 with ponderosa pine, in which the damping-off losses were 0.

In 1935, Roth (19) reported the results from a study of the effect of pH on damping-off of Norway spruce (*Picea excelsa* Link) grown in soil cultures. The amount of damping-off caused by *Corticium vagum* and *Pythium debaryanum* definitely increased from pH 3.8 to maxima between pH 6.5 and 7.0, and then decreased at pH 8.0. These results agree fairly well with the liquid-culture results but disagree somewhat with the quartz-sand culture results and American field experience.

The Effect of pH on the Growth of Douglas Fir and Ponderosa Pine Seedlings in Quartz-sand Cultures

In a preliminary study the growth of ponderosa pine and Scotch pine (*Pinus sylvestris* L.) in glycerophosphate solution B was compared with the growth in a solution containing an equal molar quantity of phosphoric acid. The seedlings were grown in quartz-sand cultures at pH 5.5 for 2½ months. There was very little difference in the amount of shoot and root growth; therefore, it was concluded that glycerophosphate solution B was satisfactory for conifer seedlings, despite some indication of superiority for the phosphoric acid indicated for Douglas fir in table 4.

When the damping-off experiments were terminated, the seedlings from the noninoculated cultures were measured for total length, and the results are tabulated in table 5 and summarized in figure 3.

As shown in table 5, the growth of Douglas fir was fair in the one series that was run at pH 2.5. The growth between pH 3.5 and 8.5 was good in all series with solution B, but the results were so variable that it was impossible to establish definite maxima. In glycerophosphate C there was less growth in most of the series with both species than in solution B. The most interesting part of the growth results is the large amount of growth that occurred in both species, in solution B at pH 8.5.

The Effect of Al-ion on Damping-off of Douglas Fir and Ponderosa Pine Seedlings in Quartz-sand Cultures with pH Held Constant

The seedlings of both Douglas fir and ponderosa pine in the quartz-

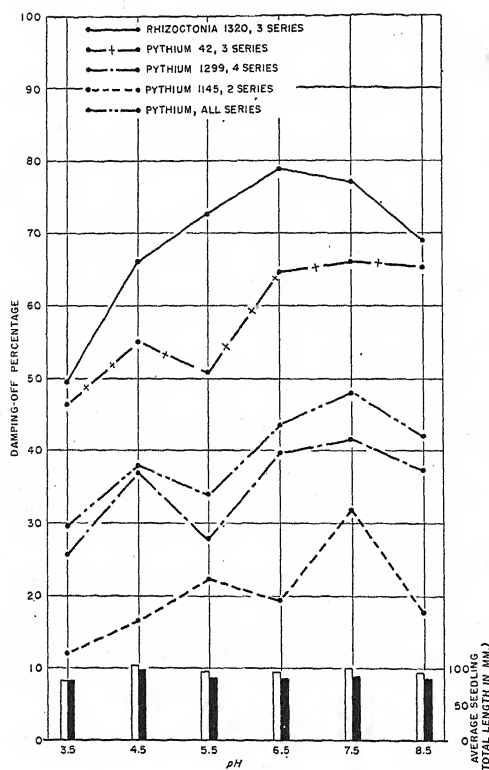


FIG. 3. Sand-culture experiments summarized; damped-off seedlings as percentages; total length of noninoculated seedlings in mm. The shaded bars represent total lengths of ponderosa pine, and the unshaded bars of Douglas fir.

sand cultures were fed with glycerophosphate solution B. The Al-ion concentrations used, which were obtained by the addition of aluminum sulphate, were 0.0005M, 0.00005M, and 0.000005M. The reactions of the solutions were adjusted to pH 3.5 and 6.5 with 0.1N HCl. The seedlings

TABLE 5.—*The growth of Douglas fir and ponderosa pine seedlings in the non-inoculated cultures in the quartz-sand series*

Series and solution	Host	Average number of seedlings	Average total length in mm. at indicated pH						
			2.5	3.5	4.5	5.5	6.5	7.5	8.5
Glycerophosphate B:									
2	Douglas fir	17	53	92	102	102	104	100	112
7	“ “	21	79	113	98	100	107	108
10	Ponderosa pine	17	83	112	87	85	106	115
			.						
Glycerophosphate C:									
14	Douglas fir	18	76	93	86	78	89	61
17	Ponderosa pine	20	80	86	86	88	71	56

were inoculated, according to the method previously described, when they reached the seed-coat-shedding stage. The number of inoculated seedlings in the cultures varied from 11 to 15. The results are given in table 6. The cultures were run simultaneously.

TABLE 6.—*Relation of damping-off of Douglas fir and ponderosa pine seedlings to the Al-ion concentration in quartz-sand cultures at pH 3.5 and 6.5*

Series	Host	Isolates	pH	Damping-off percentage at indicated aluminum-sulphate concentration			
				None	0.000005M	0.00005M	0.0005M
1	Douglas fir	Pythium 1140	3.5	7	14	0	21
5	" "	" 42	3.5	36	9	27	14
9	" "	" 1299	3.5	0	15	7	0
13	" "	Rhizoctonia 1320	3.5	29	13	23	20
3	Ponderosa pine	Pythium 1140	3.5	14	72	40	14
7	" "	" 42	3.5	72	40	47	33
11	" "	" 1299	3.5	53	13	29	7
15	" "	Rhizoctonia 1320	3.5	7	71	93	20
2	Douglas fir	Pythium 1140	6.5	47	43	57	27
6	" "	" 42	6.5	100	100	80	100
10	" "	" 1299	6.5	47	67	21	40
14	" "	Rhizoctonia 1320	6.5	85	73	93	79
4	Ponderosa pine	Pythium 1140	6.5	36	93	100	100
8	" "	" 42	6.5	91	93	87	92
12	" "	" 1299	6.5	100	100	100	100
16	" "	Rhizoctonia 1320	6.5	86	93	100	62

The relation of pH to damping-off of Douglas fir and ponderosa pine seedlings was again evident in the results from the experiment on the effect of the Al-ion on damping-off in quartz-sand cultures. In every one of the 32 possible comparisons of cultures that differed from each other by pH only, the damping-off percentages were definitely higher at pH 6.5 than at pH 3.5. The aluminum concentrations at pH 6.5 had no apparent effect on the damping-off, and none of the series at pH 3.5 showed a consistent relationship between damping-off and aluminum concentration. The only apparent effect of the aluminum was at the highest concentration, and at that only at the lower pH and mainly in the pine. On transformation of the percentages (3) to $\sqrt{x + \frac{1}{2}}$, a special analysis of variance in the results at pH 3.5, under the guidance of A. E. Brandt of the Soil Conservation Service, indicated a barely or doubtfully significant effect for 0.0005M aluminum in decreasing damping-off when both host species were considered together. Although the results indicated that the hydrogen ion had a much greater effect on damping-off than the aluminum ion, it is recognized that further experimental work, including tests of 0.0005M and also some stronger aluminum concentrations at pH 3.5 and also at one of the somewhat higher pH values likely to be found in seedbeds treated with aluminum sulphate (e.g., 4.3) is needed before an independent or additive effect of aluminum can be eliminated from the possibilities.

The Effect of the Al-ion on the Growth of Douglas Fir and Ponderosa Pine Seedlings in Quartz-sand Cultures with pH Held Constant

The roots of the seedlings were not killed by any of the aluminum concentrations used at pH 3.5 and 6.5. Measurements of the survivors in the inoculated series indicated that the roots had been definitely retarded but not seriously injured by 0.0005M aluminum sulphate at pH 3.5. It is suspected that this concentration of aluminum is practically the strongest one that can be tolerated by the hosts used in this experiment. There were not sufficient survivors at pH 6.5 for measurements. It was observed that the root hairs on both species were much more abundant in the 0.0005M than in the 0.00005M or 0.000005M concentrations of aluminum sulphate.

DISCUSSION

Field experience with pine and spruce on soils differing in acidity is that the heaviest losses occur at approximately pH 8, and that severe losses from *Pythium* or *Rhizoctonia* are rare with pH values of 5 or less. The writer's experiments on the growth of these fungi in solution B, his liquid-culture experiments on damping-off of Douglas fir, and Roth's experiments with spruce on a single soil the pH of which was artificially varied both upward and downward, agree with this field experience at low pH values but not at high pH values, the fungus activity in these experiments having been less at pH values above 7 than at those below. The writer's sand-culture experiments on the other hand have agreed with the field experience at the higher pH values, but have shown more damping-off at the low pH values than has been observed under natural conditions. The foregoing inconsistencies cannot be reconciled on the basis of present information; the differences in the composition of the culture solutions offer no obvious clue. The following suggestion may, however, be worth offering: The inoculum in the experiments was placed very near the seedlings; in the sand, where it was not actually immersed, it perhaps retained its original favorable pH for a sufficient time to enable the fungus to make the short jump to the seedling through a medium too acid or too alkaline to allow good independent growth. In regular seedbeds the seedlings first attacked constitute starting points of favorable pH from which the fungus may similarly spread to nearby seedlings even though the pH of the intervening soil is not entirely favorable to the fungus. The especially heavy damping-off at pH values well above the best growth range of the fungus may be due in part to decreased resistance of the host. There is also the possibility that the pH of the liquid films in the sand cultures differed from that of the drainage water (the basis on which the pH of the sand cultures was designated) or that hyphae passing through sand are not immersed in the soil solution at all points.

The failure of aluminum-ion concentration to affect decisively the amount of damping-off, when pH was held constant, indicates that the

efficacy of aluminum sulphate in controlling damping-off and subsequent root rot is attributable at least mainly, to its low pH rather than to the aluminum as such. The failure is of particular interest in view of the fact that 2 of the *Pythium* isolates used were from the Monument, Colorado, nursery, at which aluminum sulphate has been particularly successful in controlling not only damping-off but a subsequent root rot with which *Pythium* was the most prominent associate. The seed of both the host species in the experiment came from supplies employed at that nursery.

SUMMARY

A study was made of the effect of the H-ion and Al-ion concentrations on the damping-off disease of conifer seedlings and on the growth of damping-off fungi.

The use of sodium glycerophosphates as a phosphate source produced a nutrient solution that was stable in the presence of Fe- and Al-ions from pH 2.5 to 8.5. The solution was satisfactory for the growth of the fungi and the hosts used in the experiments.

The amount of growth produced by the *Pythium* isolates in liquid culture was zero at pH 2.5 and 3.5, increased to maxima at pH 5.5 and 6.5, and at pH 8.5 it was again zero in all except one series. Chlamydo-spore and oospore production was definitely less at pH 4.5 than at 6.5, when sugar was not present.

The amount of growth produced by the *Rhizoctonia* isolates in liquid culture was zero at pH 2.5, very small at pH 3.5, definitely more at pH 4.5, 5.5 and 6.5, then less at 7.5, and very small at 8.5. The amount of sclerotia was zero at pH 3.5 and 4.5, more at pH 5.5 and 6.5, and zero at pH 7.5 and 8.5.

The amount of damping-off of ponderosa pine and Douglas fir grown in liquid cultures, caused by *Pythium* and *Rhizoctonia* isolates, was lowest at pH 2.5 and 3.5, increased to maxima at 6.5, or less often 5.5, and then decreased at 6.5 or 7.5.

The relationship between pH and damping-off was not so consistent in the quartz-sand cultures as in the liquid cultures. The amount of damping-off was low in all the series run at pH 2.5. The damping-off maxima occurred in the series as follows: 1 at pH 3.5, 2 at 4.5, 2 at 5.5, 3 at 6.5, 4 at 7.5, and 2 at 8.5. There were 2 maxima in 2 of the series.

Aluminum showed only a barely or doubtfully significant effect on damping-off in sand cultures at pH 3.5 with a 0.0005M concentration of aluminum sulphate. The aluminum concentrations showed no effect in the series at pH 6.5. Both with and without aluminum, the damping-off percentages were definitely higher at pH 6.5 than 3.5.

The H-ion concentration at pH 2.5 was practically the lower limit for the roots of the hosts in the liquid and the quartz-sand cultures. The growth of the seedlings was excellent from pH 3.5 to 6.5, but many cultures showed definite decreases, especially in root growth, at pH 2.5, 7.5 and 8.5.

The strongest concentration of aluminum sulphate used in the experiment, which was 0.0005M, was near the limit for the roots of the hosts.

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FACTORS AFFECTING SPORE GERMINATION AND GROWTH OF UROCYSTIS OCCULTA IN CULTURE

LEE LING¹

(Accepted for publication February 1, 1940)

INTRODUCTION

As part of an investigation of the factors affecting the development of flag smut of rye, caused by *Urocystis occulta* (Wallr.) Rab., the writer studied some of the factors affecting the germination of chlamydospores and the growth of the fungus on artificial media.

Kühn (11), Wolff (20), Brefeld (4), McAlpine (13), and Stakman, Cassell, and Moore (16) described the essential features of spore germination. The last-named authors also made some observations on the effects of temperature on the rapidity and percentage of spore germination and showed that germination was stimulated by benzaldehyde. In addition, they described the nuclear behavior from chlamydospore germination to their formation in the host.

Little was known, however, about the effect of factors other than temperature on spore germination, and nothing was known about growth on artificial media and the factors affecting it. Neither has it been known whether the haplophase could be grown in culture, nor whether the sporidial branches might function as true sporidia under appropriate conditions. The studies reported in this paper therefore were made.

METHODS

Experiments were made chiefly in Syracuse dishes, though in some cases the hanging drop method was used also. Chlamydospores were sown on the surface of the medium simply by breaking the sori and shaking the diseased leaves. In studying the growth of the fungus in culture, 250 cc. Erlenmeyer flasks containing 50 cc. of medium were used. Because of the very slow growth of the fungus, cultures were usually grown for 10 to 12 weeks before final notes were taken. Except in the special study of temperature relations, all cultures were incubated at a temperature fluctuating between 17° and 21° C. Each experiment comprised 3 or 4 replications, and figures presented in the tables on germination and growth rate are an average of these replicates.

¹ A condensed portion of a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted by the University of Minnesota, June, 1937. The writer acknowledges his indebtedness to Dr. E. C. Stakman, Dr. J. J. Christensen, and Mr. M. B. Moore for suggestions and help.

FACTORS AFFECTING SPORE GERMINATION

Effect of Temperature

Stakman, Cassell, and Moore (16), in their cytological study of *Urocystis occulta*, made germination tests at temperatures ranging from 9.5° to 28° C. They found that early germination was best at about 15° C., but 24° C. was more favorable for subsequent development of germ tubes. The writer obtained similar results (Table 1).

TABLE 1.—Effect of temperature on germination of spores of *Urocystis occulta* in benzaldehyde solution (3:2,000,000)

Temperature, in degrees C.	Percentage of germination after stated number of hours						
	36	48	72	96	120	144	168
5	0
10	3	26	++ ^a
15	3	36	++
20	31	++
25	2	5	++
30	0

^a ++ indicates additional germination and extensive growth of hyphae whenever actual counts were not made.

It will be noted that a small percentage of spores germinated in 36 hours at 15° C., but 48 hours usually is required at 15°–20°. At lower or higher temperatures more time is required for germination, although the final percentage of germination may be high. As rapidity of germination is important in enabling the pathogen to cause infection in nature, the effect of soil temperature on the percentage of infection (12, 17) probably is explicable on this basis.

The Effect of Substratum and Chemical Stimuli

Several liquid and solid media and various chemicals were tested with respect to their effect on germination of spores. They are grouped into 3 classes: (1) Liquid media—Distilled water, tap water, sterile soil water, sugar solutions of various concentrations; (2) Agar media—Plain agar, plant agar plus a trace of benzaldehyde, soil extract, rye extract, potato dextrose, 3 per cent malt extract, maltose of various concentrations; (3) Chemicals and plant tissues—Benzaldehyde, various organic acids, host-plant tissue, non-host-plant tissue.

Although Brefeld (4) and McAlpine (13) stated that *Urocystis occulta* germinated readily in water, the writer was unable to obtain good germination in either distilled or tap water. In distilled water the highest percentage of germination ever observed was approximately 25 per cent, and various sugar solutions did not materially increase the amount of germination. Therefore, the effects of various stimuli were studied.

Noble (14) reported that benzaldehyde, 3:2,000,000 was the most effective chemical in stimulating germination of spores of *Urocystis tritici* Koern. Likewise, Stakman, Cassell, and Moore (16) found that presoaking chlamy-

dospores of *U. occulta* in benzaldehyde solution of the same concentration for 16 hours stimulated abundant germination. Results of the present study are in agreement (Table 2) but germination was equally good in the benzaldehyde solution itself, and the range of concentration favorable for germination seemed to be wider than that given by Noble.

TABLE 2.—Effect of medium and temperature on germination of spores of *Urocystis occulta*

Medium	Temperature in degrees C.	Percentage germination after stated number of hours ^a							
		48	72	96	120	144	168	192	216
Distilled water	8	few	5	19
	16-18	2	9	15	++
	23-25	1	2	4	17	—
	27.5	0
Sterile soil water	8	1	9	+
	16-18	7	36	++
	23-25	1	24	—
	27.5	0
Sucrose solution, 2 per cent	8	0
	16-18	1	9	++
	23-25	1	—
	27.5	0
Benzaldehyde solution, 3:2,000,000	8	3	13	30	95
	16-18	41	++
	23-25	4	14	++
	27.5	0

^a The following symbols were used to denote the degree of germination whenever counts were not made:

— None or very few spores germinated.

+ Moderate germination.

++ Good germination and extensive growth of hyphae.

Tissues of rye and other plants were inconsistent in effects when added to water. In many cases they greatly increased the percentage of germination, but sometimes they were entirely ineffective. As the results were so erratic in repeated tests, no general conclusion can be drawn and the detailed data are not given.

Organic acids, such as malic acid, lactic acid, citric acid, and oxalic acid, were added in different amounts to distilled water, in an attempt to induce better germination, but without success.

On agar media, *Urocystis occulta* in most cases failed to germinate. Of the media used, germination was observed only on the following: maltose agar at concentrations of M/6 to M/8, plain agar plus a trace of benzaldehyde, and oatmeal agar. In all cases the percentage of germination was extremely low, usually not over 5 per cent. It seems, therefore, that more wetting is necessary than is likely to occur on agar.

The results of spore germination in distilled water, sterile water, 2 per cent sucrose solution, and benzaldehyde solution (3:2,000,000), at 4 temperatures are summarized in table 2. In this experiment the temperature

fluctuated 1 or 2 degrees. It is noteworthy that the medium affected not only the final percentage but also the speed of germination. Spores germinated most rapidly in benzaldehyde solution at all temperatures within the range for germination, and next most rapidly in sterile soil infusion. Soil water is, of course, the natural medium for germination, and it, therefore, seems significant that soil infusion is so favorable. The lower temperature limit for germination seems to be about 8° C., but after 8 days a very uniform germination of about 95 per cent was observed at that temperature in benzaldehyde solution.

The Effect of Light

To determine the effect of light on germination, Syracuse dishes containing spores in 4 different liquid media were kept under the following conditions: (1) in direct sunlight; (2) in diffuse light; and (3) in complete darkness. Results given in table 3 indicate that darkness is generally most favorable for germination, except in benzaldehyde solution, where diffuse light was about equally favorable. In every case, however, direct sunlight decreased the percentage of germination somewhat. Temperature may have had some effect, as it was unavoidably higher in direct sunlight than under other conditions. Nevertheless, it appears that the spores are well adapted to germination in darkness; hence, in the soil.

TABLE 3.—*Effect of light on germination of spores of Urocystis occulta in four liquid media*

Medium	Light condition	Percentage of germination after stated number of hours ^a						
		48	56	72	96	120	144	168
Distilled water	Sunlight	0	0	few	—	0
	Diffuse light	0	1	3	8	+
	Darkness	3	12	14	19	+
Sterile soil water	Sunlight	1	34	—
	Diffuse light	5	39	++
	Darkness	13	59	++
Sucrose solution, 2 per cent	Sunlight	few	3	4	—
	Diffuse light	few	1	4	—
	Darkness	3	4	23	+
Benzaldehyde solution, 3: 2,000,000	Sunlight	21	22	++
	Diffuse light	31	38	++
	Darkness	29	34	++

^a The following symbols were used to denote the germination process, whenever actual counting was not made:

— None or very few additional spores germinated.

+ Additional germination.

++ Additional germination and extensive growth of hyphae.

The Effect of Hydrogen-ion Concentration

Germination tests were made in benzaldehyde solution (3:2,000,000), which was adjusted with NaOH and HCl to different hydrogen-ion concen-

trations, determined electrometrically with a quinhydrone electrode. The tests were made in Syracuse dishes, in triplicate. Germination occurred between pH 5.08 and 8.95, with an optimum at pH 6.86, 70 per cent of the spores germinating within 72 hours at this concentration. There was no germination at pH 3.80, but 5 per cent of the spores still germinated at pH 8.95. Obviously, therefore, spores can germinate in soils differing greatly in hydrogen-ion concentration.

GENERAL CHARACTERS OF THE FUNGUS ON ARTIFICIAL MEDIA

Single Chlamydospore Cultures

As concerns saprophytism, species of the genus *Urocystis* have not received as much attention as certain species of *Ustilago* and *Sphacelotheca*. Anderson (1) stated that *Urocystis cepulae* Frost can be isolated and grown on many culture media and on soil. Later, Blizzard (2) made an extensive study of the cultural characteristics of the mycelium, and Walker and Wellman (19) have investigated the relation of temperature to its growth. Kniep (10) succeeded in growing *Urocystis anemones* (Pers.) Wint. in liquid media and observed the complete life cycle, from chlamydospores to chlamydospores, in 0.1 per cent malt extract. Attempts by Verwoerd (18) to grow *Urocystis tritici* on various media were not successful, however.

In general, only the haplophase of smuts grows extensively as a saprophyte, the dicaryophase usually being restricted to the host plant and the diplophase to the chlamydospores and young promycelia, except in a few cases in which the sporidia, and, consequently, monosporidial lines, are diploid. The so-called sporidia in *Urocystis occulta* do not behave like true sporidia because they do not become abjointed; hence, the only possibility of obtaining haploid lines would be to cut single sporidial branches from the promycelia and propagate them. The writer made attempts to obtain haploid lines in this manner. Cultures also were derived from single chlamydospores. As sporidial branches may fuse and germinate on the promycelia, however, the nuclear condition of cultures resulting from the single diploid chlamydospores might be somewhat variable.

Although the percentage of germination of *Urocystis occulta* on agar media was extremely low, the mycelium grew well. Pure cultures were obtained readily from spores germinated in benzaldehyde solution and later transferred to malt-extract agar, but single-spore cultures were obtained with difficulty. The following method, however, was fairly satisfactory. Presoaked spores were scattered on a drop of maltose agar and those just starting to germinate were picked up individually and transferred to separate agar drops, on which colonies subsequently developed. Transfers were then made to agar in tubes or flasks. As all the colonies secured in this way behaved more or less alike, only one was used throughout the later experiments.

The growth of the fungus in culture was very slow. On rich media, like potato-dextrose and maltose agars, the colonies were very compact, thick,

convex, rugose, and white or with a faint buff-brown tinge in the center.² On less suitable media the colonies were thinner, flat, smooth, often zonate, and entirely white. Other variations in cultural characteristics induced by different conditions will be described in connection with the discussion of environmental effects.

In liquid media the mycelium formed numerous, small, willow-catkin-like balls. If the mycelial web became attached to the wall of the flasks or to something that could be used as a support, an aerial growth of compact white mycelium resulted.

Sectoring was observed in vigorously growing colonies (Fig. 1), though

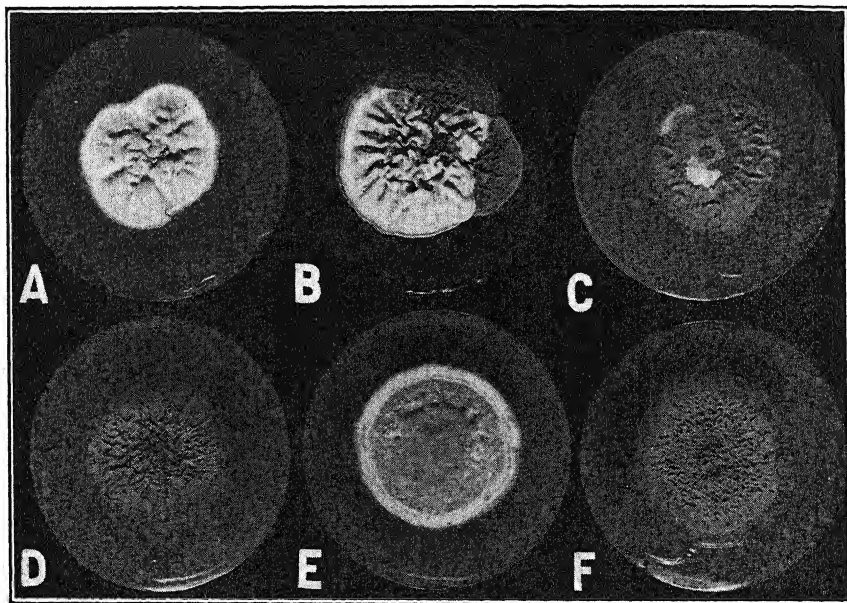


FIG. 1. Variation in growth form of *Urocystis occulta*. A. Original type of monosporous culture. B. Same, showing sectors. C. Monosporidial culture. D to E. Isolates from sectors arising from monosporous culture.

not so frequently as in *Ustilago zeae* (Beckm.) Ung. and certain other species. The most common type of sectors were light buff, flat and felty, and with considerable aerial mycelium. Their growth usually was faster than that of the original type.

The saprophytic mycelium was either binucleate or multinucleate, mostly 1.0 to 2.6 μ in diameter, and often with chains of intercalary chlamydospore-like bodies. The cytological evidence and the formation of these chlamydospore-like bodies indicate strongly that the dicaryotic mycelium is capable of considerable development on artificial media, although the dicaryophase of most smuts usually is restricted largely to the host plant. That this phase of *Urocystis occulta* can grow saprophytically, at least to

² Colors given are those described in Ridgway's Color Standards (15).

some extent, is shown also by the work of Stakman, Cassell, and Moore (16), who illustrate fairly long hyphae with paired nuclei.

The sectoring observed in monosporous cultures, therefore, is not necessarily evidence of mutation. It may have resulted from nuclear dissociation; but in any case it is clear that several biotypes may arise from monosporous cultures, as is true of many other smut fungi.

"Monosporidial" Cultures

Stakman, Cassell, and Moore (16) showed that usually there is a single large nucleus in the very young promycelium. By successive divisions a number of nuclei equal to the number of sporidial branches are then produced. Normally one nucleus passes into each sporidial branch, after which the branches fuse in pairs and a dicaryotic hypha grows from one of each fusion pair. The sporidia, however, have never been observed to become detached from the promycelium; and the condition is roughly analogous to self-pollination in higher plants, except when sporidial branches on different promycelia fuse. Consequently, the opportunity for variation resulting from hybridization seems somewhat limited. To obtain further information regarding the sexual nature of the sporidial branches and to find out whether new biotypes might arise through crossing, attempts were made to isolate and study monosporidial lines.

In 1935 several hundred sporidial branches were cut from germinating spores by the method described by Dickinson (8), but only two grew into colonies. All others became vacuolated and failed to grow, probably because no definite septum is formed between the sporidium and the promycelium; consequently the cytoplasm may flow out from the cut end of a sporidial branch and thus cause death. The method of breaking off the promycelial branches by shaking germinating spores in water, as described by Clyde Christensen (6) for isolating haploid lines of *Ustilago tritici*, seemingly was not successful, as the resulting colonies resembled those of single-chlamydospore origin and most probably were not haploid.

The two monosporidial lines obtained differed from the single-chlamydospore cultures in having a buff-pink color and a faster and more spreading growth. Inoculations on rye seedlings, using the method described by Flor (9), with the two monosporidial lines alone and in combination failed to cause infection, although positive results were obtained with a mass culture. The sexuality of these two lines therefore could not be determined.

FACTORS AFFECTING GROWTH ON MEDIA









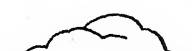

As a preliminary to determining the effect of environmental factors on the development of *Urocystis occulta* in rye plants, studies were made of factors affecting growth of monosporous cultures on artificial media.

The effects of environment on the growth of fungi have been recorded in various ways. The relative advantages of linear measurements of colonies and of the dry-weight determination method was fully discussed by Brown

(5), who concluded that the former is best for exploratory work. This method therefore was used. Solid media were used in these studies because the cultural characters are more distinctive on them than on liquid media.

Effect of Temperature

Single chlamydospore cultures were grown on potato-dextrose and on 3 per cent malt-extract agars at temperatures ranging from 5° to 25° C. The effect of temperature was essentially the same on the two media used. Growth occurred at all the temperatures tried, the optimum being near 20° C. This is in agreement with the statement of Stakman, Cassell, and Moore (16) that the optimum temperature for growth of *Urocystis occulta* in the host is higher than for spore germination. The relative size and topography of the colonies at different temperatures is shown in figure 2.

Temp., in Deg. C.	Diagram of colonies and diameter in mm. <i>La</i>	
	Potato-dextrose agar.	3% Malt agar.
50	15 	6 
10	23 	19 
75	43 	26 
20	44 	40 
25	27 	22 

La Results are based on cultures twelve weeks old, grown in triplicate flasks.

FIG. 2. Effect of temperature on growth of *Urocystis occulta* on agar media.

Hydrogen-ion Concentration

Potato-dextrose agar was adjusted by the addition of NaOH and HCl after sterilization and before solidification, so that a series of media with

various reactions was obtained. The pH of each was determined colorimetrically with a comparator following the methods described in detail by Clark (7). The organism grew over a rather wide range of hydrogen-ion concentrations, even on a highly alkaline agar with a pH value of 9. The optimum concentration for growth is about pH 6.2 (Table 4). No appreciable effect on the growth form was observed.

TABLE 4.—*Effect of hydrogen-ion concentration on growth of Urocystis occulta on potato-dextrose agar*

Initial pH	Diameter of colonies in mm. ^a
5.0	39
5.6	42
6.2	45
6.8	40
7.6	34
8.4	27
9.0	25

^a Average of triplicate colonies, 10 weeks old.

Nutrition

Carbohydrates and Minerals. Growth was first studied on agar media containing different concentrations of various carbohydrates. The media were steamed without pressure for 30 minutes on 3 consecutive days to avoid hydrolysis. The results are summarized in table 5. Since carbohydrates were used alone, the fungus in reality was growing under conditions of starvation with respect to nutritive elements other than carbon, but the results give indications of the relative value of these carbohydrates as a source of carbon.

In general, there was only sparse growth on these media, except maltose, on which the fungus apparently grew normally, probably because of small amounts of impurities in the maltose used. Both rhamnose and levulose appeared to be unfavorable, although the reactions to them were entirely different. Growth was best on the higher concentrations of rhamnose so this sugar must have some nutritive value, although not in low concentrations. On the contrary, levulose appeared to be deleterious to the fungus, as it grew at the lowest concentration only and growth was entirely inhibited at higher concentrations. Brannon (3) reported a similar case with peas.

Later, the fungus was grown on a series of agar media in which certain compounds in the basic formula were either increased, omitted, or replaced by other compounds. The basic formula contained practically all the nutritive elements essential for plant growth. The results are presented in table 6, in which the relative order of growth also is given to facilitate comparison between the media. Phosphorus, nitrogen, and magnesium seemed to be indispensable for growth, since the omission of any one had an appreciably unfavorable effect. A 5-fold increase in the quantity of KH_2PO_4 was deleterious, but an increase in $\text{Ca}(\text{NO}_3)_2$ or MgSO_4 had very little influence. Among the carbohydrates used, sucrose failed to promote as good growth as

TABLE 5.—*The growth of monosporous cultures of Urocystis occulta on agar plus various concentrations of carbohydrates*

Kind and concentration of carbohydrate		Initial pH	Diameter of colonies in mm. ^a	Growth form
Rhamnose	m/18	5.6	Trace	Very thin and effuse, almost colorless
	m/7	5.4	Do	Do
	m/4	5.4	20	Do
Dextrose	m/10	5.4	27	Very thin, effuse, whitish
	m/5	5.4	29	Do
	m/2.5	5.2	23	Very thin, effuse, almost colorless to whitish
Levulose	m/10	4.8	19	Very thin, effuse, whitish
	m/5	4.8	No growth	
	m/2.5	4.8	Do	
Sucrose	m/20	6.0	25	Thin, white, colorless toward the margin
	m/10	6.0	29	Do
	m/5	5.9	28	Do
Maltose	m/20	5.0	36	Thick, faint walnut-brown in center, white toward margin
	m/10	5.0	41	Thick, white
	m/5	5.2	36	Very thick, white
Inulin	m/56	5.6	27	Thick, white, almost colorless toward margin
	m/28	5.5	23	Do
	m/14	5.4	30	Do
Wheat starch	1.5%	6.0	24	Thin, effuse, white, colorless toward margin
	3.5%	5.9	26	Do
	7.0%	14	Do
Check ^b		4.8	45	Very thick, white

^a Results are based on cultures 11 weeks old, grown in triplicate flasks.

^b A synthetic medium consisting of: Maltose, 25 g.; dextrose, 10 g.; asparagin, 1 g.; Ca(NO₃)₂, 3 g.; K₃PO₄, 1 g.; MgSO₄, 1 g.; FeCl₃, .02 g.; agar, 15 g.; and distilled water, 1000 cc.

dextrose, while maltose appeared to be superior to dextrose. Mannite apparently was not available to the fungus, as no growth was observed on the medium containing it. When the amount or the source of carbon, dextrose in this case, was increased, the growth was considerably improved. As a whole, the fungus grew best on potato-dextrose agar.

Plant Juices. In an attempt to stimulate growth, agar media containing expressed juice of sugar beet roots, tubers of dahlia and potato, and rye plants were tested alone and in combination with a synthetic medium. Potato dextrose was also included as an extra check. In general, plant juices alone did not promote as good growth as the synthetic medium. Sugar beet juice, however, induced a unique type of growth, characterized by strongly convex, very smooth, and thick colonies. A comparison between the synthetic media with and without plant juices showed that it was difficult to

TABLE 6.—*The growth of monosporous cultures of Urocystis occulta on synthetic agar media in various modifications*

Modifications of basic formula ^a	Diameter of colonies in mm. ^b	Growth form	Relative order of growth ^c
No change	28	Smooth, flat, white	4
K ₂ SO ₄ for KH ₂ PO ₄	28	Very thin, flat, pulveraceous on surface, zonate, white, colorless toward margin	7
5 times KH ₂ PO ₄	15	Very thin, flat, pulveraceous on surface, white	8
No Ca(NO ₃) ₂	18	Very thin, flat, pulveraceous on surface, white, colorless toward margin	9
5 times Ca(NO ₃) ₂	29	Very thin, flat, pulveraceous on surface, zonate, white	5
Na ₂ SO ₄ for MgSO ₄	16	Very loose mycelial mat, colorless	10
5 times MgSO ₄	26	Smooth, flat, white	4
5 times dextrose	42	Slightly velvety, flat, white	2
Sucrose for dextrose	23	Smooth, flat, white	6
Maltose for dextrose	35	Smooth, flat, slightly zonate, white	3
Mannite for dextrose	0		
Potato-dextrose	40	Thick, convex, rugose, zonate toward margin, white	1

^a Basic formula: KH₂PO₄, 1.22 g.; Ca(NO₃)₂, 0.42 g.; MgSO₄, 0.90 g.; FeSO₄, trace; dextrose, 10 g.; distilled water, 1000 cc.

^b Results are based upon cultures 11 weeks old, grown in flasks in quadruplicate.

^c Best growth is designated as 1.

conclude where the best growth occurred, considering both the diameter and the thickness of colonies, but the writer is inclined to think that plant juices did not accelerate the growth rate appreciably.

DISCUSSION

The primary object of the present investigation was to obtain information basic to a more complete investigation of the environmental factors affecting the development of *Urocystis occulta* in susceptible and resistant lines of rye.

The pathogen is well adapted to its mode of life and parasitism. Spores germinate over a rather wide range of conditions, soil water is a good medium and darkness is favorable. Moderate to low temperatures, such as usually prevail when rye is sown in the fall in northern regions, are optimum for germination and the slightly higher but still relatively low optimum for growth is also advantageous for a pathogen of a fall-sown crop plant of northern regions.

It is becoming increasingly clear that the existence of physiologic races in the smut fungi is one of the very important factors affecting their development in different regions. The writer, therefore, attempted to find out whether there are numerous biotypes within the species, as is true of certain of the other smut fungi, and whether new ones are produced by recombinations and mutations. Two difficulties were encountered, however; the fungus

grows slowly on artificial media; and haploid lines are difficult to obtain because the so-called sporidia do not function as conidia. They do not abjoin from the promycelium but fuse *in situ* with other sporidia. As no septum is formed at their base, they are likely to die when cut off from the promycelium. Of several hundred cut off by the writer, only two grew and produced colonies. Evidently they were unisexual and of the same sex, as they did not infect rye, either singly or in combination. Nevertheless it is clear that there are biotypes, as distinct cultural lines were isolated from sectors in monosporous cultures.

Further study of the genetics of monosporous lines are desirable, but the approach necessarily must be indirect and the evidence is likely to be circumstantial. Possibly some way can be devised for obtaining haploid lines from monosporous cultures. Possibly a set of conditions can be found under which the haploid sporidial branches may grow instead of fusing. Further studies on the possible effect of growth-promoting substances in culture also are desirable.

SUMMARY

Chlamydospores of *Urocystis occulta* germinated at temperatures ranging from about 10° C. to 25° C., but not at 5° and 30°. The optimum is about 15°. Temperature affects the rapidity more than the final percentage of germination.

The substrate and certain chemicals affect both the rapidity and percentage of germination. Solid media are unfavorable. Spores do not germinate well in distilled water, tap water, and sugar solutions, but they germinate well in soil infusion. Benzaldehyde in concentration of about 3:2,000,000 has a decidedly stimulating effect. Organic acids, such as malic, lactic, citric, and oxalic, did not stimulate germination, and the effect of plant tissues was erratic.

In general, spores germinate better in darkness or diffuse light than in direct sunlight, although this may have been partly a temperature effect.

The optimum hydrogen-ion concentration for spore germination is about 6.86. There was no germination at 3.80 but 5 per cent of the spores still germinated at 8.95.

Urocystis occulta grows slowly on artificial media, the colonies of monosporous cultures attaining a maximum diameter of 45 mm. only after 10 weeks.

Sectors were produced commonly, although not abundantly, in monosporous cultures. Distinct lines were obtained by transferring from these sectors, but the reason for the sectoring is not known, as the hyphal cells were binucleate or multinucleate and various combinations of nuclei may have occurred.

Several hundred sporidial branches were cut from promycelia in attempts to isolate haploid lines, but only two were obtained. These lines differed from monosporous lines in cultural characters and they failed to infect rye, either singly or in combination.

Monosporous cultures grew on potato-dextrose and malt-extract agars at temperatures ranging from 5° to 25° C., the optimum being about 20°, which is about 5° higher than the optimum for spore germination.

The organism grew moderately well on potato-dextrose agar with initial hydrogen-ion concentrations of 5 to 9, the optimum being 6.2.

Of the solid media tried, potato-dextrose agar appeared to be the best. Many carbohydrates, with the exception of rhamnose and levulose, supported fair growth, levulose appearing toxic. Of the mineral elements, phosphorus, nitrogen, and magnesium were indispensable. Expressed juices of sugar beet, dahlia, potato, and rye did not appreciably promote growth.

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CORYNEUM BLIGHT OF ORIENTAL ARBORVITAE CAUSED BY *CORYNEUM BERCKMANII*, N. SP.¹

J. A. MILBRATH²

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INTRODUCTION

Wagener³ has recently reported a 5-septate species of *Coryneum* (*C. cardinale* Wagener), which threatens to exterminate its chief host, the Monterey cypress (*Cupressus macrocarpa*), in its native California habitat. A similar species of *Coryneum* has caused an enormous loss of Oriental arborvitae in the nurseries of the Pacific Northwest. The disease, known locally as "Berckman blight," because of its frequency on the Berckmans' variety, *Thuja orientalis* L. var. *conspicua* Berckmans, has been present for at least 10 years, but it was not until 1932 that the fungus became sufficiently well-established to cause serious losses. The disease became so severe that the majority of nurserymen could not grow plants suitable for sale. Moreover, its presence in home gardens decreased the demand for this desirable group of ornamentals. This paper presents the symptomatology of the disease, describes as the pathogen a new species of *Coryneum*, and reports control investigations.

DESCRIPTION OF THE DISEASE

Coryneum blight is characterized by the blighting of small branches, causing them to change from the normal green to a reddish-brown. At first the blighted branches are inconspicuous, but secondary infections soon appear in the surrounding foliage and render the plants unsightly. Many of the small dead branchlets fall from the plant, leaving a tangle of dead grey stems. Girdling delimits the ends of the infected areas on larger branchlets. The foliage invaded by the fungus soon turns light grey. The branchlets killed by girdling become reddish-brown in late spring when the weather becomes warm and dry. As new growth develops in blighted areas the fungus is spread by spores to the young contiguous foliage. Reinfection continues until the plant becomes so devitalized that it dies. The disease may be recognized by these various stages of browning and by the presence of the black spore pustules of the *Coryneum* (Fig. 1, A).

Primary infections occur on the young scale leaves of the terminal branchlets. Infection is facilitated by small wounds, but the fungus can enter uninjured tissue. The fungus invades and girdles small and succulent stems, but it is unable to girdle the larger stems that have become woody. It develops only small cankers on the woody stems at the base of

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² The writer wishes to acknowledge the assistance of Dr. F. P. McWhorter in conducting these investigations and in the preparation of this paper.

³ Wagener, W. W. The canker of *Cupressus* induced by *Coryneum cardinale*, n. sp. Jour. Agr. Res. [U.S.] 58: 1-46. 1939.

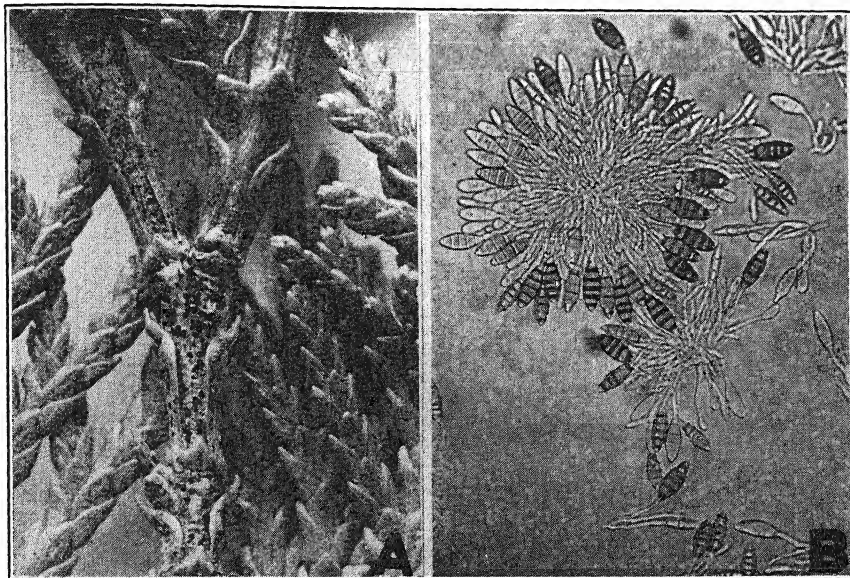


FIG. 1. A. Portion of young branchlet showing spore pustules of *Coryneum berckmanii*. $\times 3$. B. Water mount of a crushed unstained spore pustule. $\times 200$.

the blighted branchlets. Attempts to isolate the fungus from such cankers have been unsuccessful, indicating that it had become inactive. Natural infection does not occur on the large woody stems, and artificial inoculations on such stems were unsuccessful.

Sporulation is restricted to the scale leaves or the stems small enough to be enclosed by scale leaves. Spore pustules have not been observed on the small stem cankers, which develop at the base of blighted branchlets. Abundant spore formation results when the small stems are girdled (Fig. 1, A), but spore pustules form singly or in groups of 2 or 3 on the scale leaves invaded by the fungus.

NATURAL HOST PLANTS

The ornamental varieties of *Thuja orientalis* L. are the principal hosts of the *Coryneum* blight, but the columnar Italian cypress, *Cupressus sempervirens* L. var. *stricta* Ait. is occasionally affected. These are the only two species known to be susceptible. The varieties of *T. orientalis* L. found naturally infected are var. *conspicua* Berckmans, var. *beverleyensis* Rehd., var. *elegantissima* Gord, var. *compacta* Weiss and var. *stricta* Leud. This *Coryneum* has not been found on any other species of *Thuja*, or on any other conifer except Italian cypress. Many species of conifers that were grown adjacent to infected plantings of *T. orientalis* have been examined without finding indications of natural infection. When 50 *T. orientalis* were placed in a diseased planting, 39 of these plants became infected during the first 2 years. It would seem, therefore, that other related species of conifers

growing adjacent to blighted plants for several years would become diseased if they were at all susceptible. Since natural infection was not observed on any of these species of plants, and since natural infection has been more successful than artificial inoculations on the known susceptible hosts, a large series of cross-inoculations was not deemed worthwhile.

THE CAUSAL FUNGUS

Description

An examination of many specimens of the *Coryneum* and a comparison of this fungus with the descriptions of other 5-septate species of *Coryneum* indicate that this is an undescribed species; the name *Coryneum berckmanii* is proposed.

Coryneum berckmanii, sp. nov.

Acervulis conspersis, atris, erumpentibus, compactis, pulvinatis, circa 246 μ diam., a primordiis sub epidermate orientibus; stromatibus parvis, obscuris; conidiis oblongo-fusoidis, 5-septatis, 28.8 μ (24.8–36.5 μ) \times 9.9 μ (8.6–11.5 μ), loculis 4 mediis concoloribus, olivaceo-brunneis, ad cepta non vel vix constrictis, loculis extimis breviter conicis, hyalinis, non rostratis, conidiophoris erumpentibus,, multi-ramosis, septatis, hyalinis, 42.6 μ (26.6–53.2 μ) \times 2.3 μ (2.0–2.6 μ), ramis 23.3 μ (13.3–25.1 μ) \times 2.3 μ , paraphysatis. Hab. in foliis et ramulis *Thuja orientalis* L. et *Cupressi sempervirentis* L.; Oregon, Washington.

Acervuli scattered, black erumpent, pulvinate, reaching an average diameter of 246 μ , originating from a single primordium just below the epidermis, the elongating conidiophores piercing the epidermis before conidial differentiation; stromatic layer small, inconspicuous, conidia oblong-fusoid, 5-septate, 28.8 μ (24.8 μ –36.5 μ) \times 9.9 μ (8.6–11.5 μ), the four median cells concolorous, olive brown, nonconstricted or only slightly constricted at the septa, end cells short-conic, hyaline, not beaked; conidiophores for the most part many times branched, septate, hyaline, 42.6 μ (26.6 μ –53.2 μ) \times 2.3 μ (2.0 μ –2.6 μ); individual spore pedicels 23.3 μ (13.3 μ –25.1 μ) \times 2.3 μ ; paraphyses absent.

On scale-leaves and small branchlets of *Thuja orientalis* L. and *Cupressus sempervirens* L.; Oregon and Washington.

Specimens Examined

The following specimens in the mycological herbarium of the Botany Department of the Oregon State College, Corvallis, Oregon, were examined:

On Thuja orientalis.—11,100 (types), Portland, Oregon, April, 1937; 11,101, Salem, Oregon, April, 1937; 11,102, Salem, Oregon, April, 1937; 11,108, Eugene, Oregon, May, 1937; 11,111, Eugene, Oregon, May, 1937; 11,112, Hillsboro, Oregon, May, 1937; 11,113, Corvallis, Oregon, May, 1937; 11,149, Corvallis, Oregon, January, 1938; 11,151, Salem, Oregon, January, 1938; Corvallis, Oregon, February, 1939.

On Cupressus sempervirens.—11,158, Gresham, Oregon, February, 1938; 11,162, Tigard, Oregon, March, 1938.

Portions of the type collection are deposited in the herbaria of the United States Department of Agriculture in Washington, D. C., The New York Botanical Garden, and the Farlow Herbarium of Harvard University. The perfect stage of the fungus has not been found on any of the specimens examined, and has not been observed in culture.

Comparison with Other Species

Only 4 of the 10 species of *Coryneum* described on conifers, belong to the group having 5-septate spores. These are *Coryneum juniperi* All. described from Bavaria,⁴ *C. calosporum* Naumov on the bark of *Picea excelsa* in Russia,⁵ *C. abietinum* Ell. and Ev. on *Abies* sp. from Newfoundland,⁶ and *C. cardinale* Wagener on *Cupressus* in California.⁷ The longer conidiophores, the smaller conidia and the spreading stroma of *C. juniperi* differentiates this species from *C. berckmanii*. The conidia of *C. calosporium* are longer and broader than those of *C. berckmanii*. The acervuli of *C. calosporium* have paraphyses. *C. abietinum* has longer and narrower spores than *C. berckmanii* and the conidiophores of the former are longer. According to Wagener⁷ the three above species are not considered pathogenic. A comparison between *C. cardinale* and *C. berckmanii* is therefore especially significant, these being the only 5-septate forms known to cause important diseases. *C. berckmanii* differs from *C. cardinale* in that the latter has a loculate stroma, a different type of acervulus, smaller spores, pseudoparaphyses, and different characteristics in culture (Fig. 2). *C. cardinale* infects mature woody stems, while *C. berckmanii* develops only in foliage and young stems.

Cultural Characteristics

Coryneum berckmanii grows readily on potato-dextrose agar, and forms a colony of regular outline and compact texture. The surface is usually smooth, but some colonies may become uneven because of zonate bands. The color is pale pink to salmon pink in the central portion, fading to white at the advancing edge. The colony grows quite rapidly at room temperatures until it reaches a diameter of 20 mm.; then the rate of growth decreases, eventually stopping after the colony reaches a diameter of 35-40 mm. The growth at 50-65° F. is almost twice that at room temperature (75-80° F.), and continues until the entire surface of the plate becomes covered. The surface of colonies growing in partly closed containers, such as a plugged flask, becomes studded with raised clumps of pink mycelium. These do not develop until the expansion of the colony is limited by the edges of the container. Growth similar to that described above occurs on several Difco-bacto agars, viz., potato-dextrose, cornmeal, bean pod, and lima-bean. On prune agar, however, the growth was very sparse attaining only 8 mm. in 2 weeks, the mycelium was completely submerged in the medium and the colony became olive brown.

⁴ Allescher, A. Die Pilze Deutschlands, Oesterreichs, und der Schweiz. VII Abtheilung. Fungi Imperfecti: Gefarbt-sporige Sphaerioideen, sowie Nectrioideen, Leptostromaceen, Excipulaceen und Familien der Ordnung der Melanconieen. In Rabenhorst, L., Kryptogamen-Flora von Deutschland, Oesterreich, und der Schweiz, Aufl. 2, Bd. 1, Abt. 7, 1072 pp. E. Kummer, Leipzig. 1903.

⁵ Naumov, N. Champignons de L'Oural. Ural'skoe Obsch. Estestvoznaniia v. Ekaterinburgie Zap. (Bull. Soc. Oural. d'Amis des Sci. Nat.) 35, 48 pp. 1915.

⁶ Ellis, J. B., and B. M. Everhart. New species of fungi from various localities. Acad. Nat. Sci. Phila. Proc. 1894: 322-386. 1895.

⁷ See footnote 3.

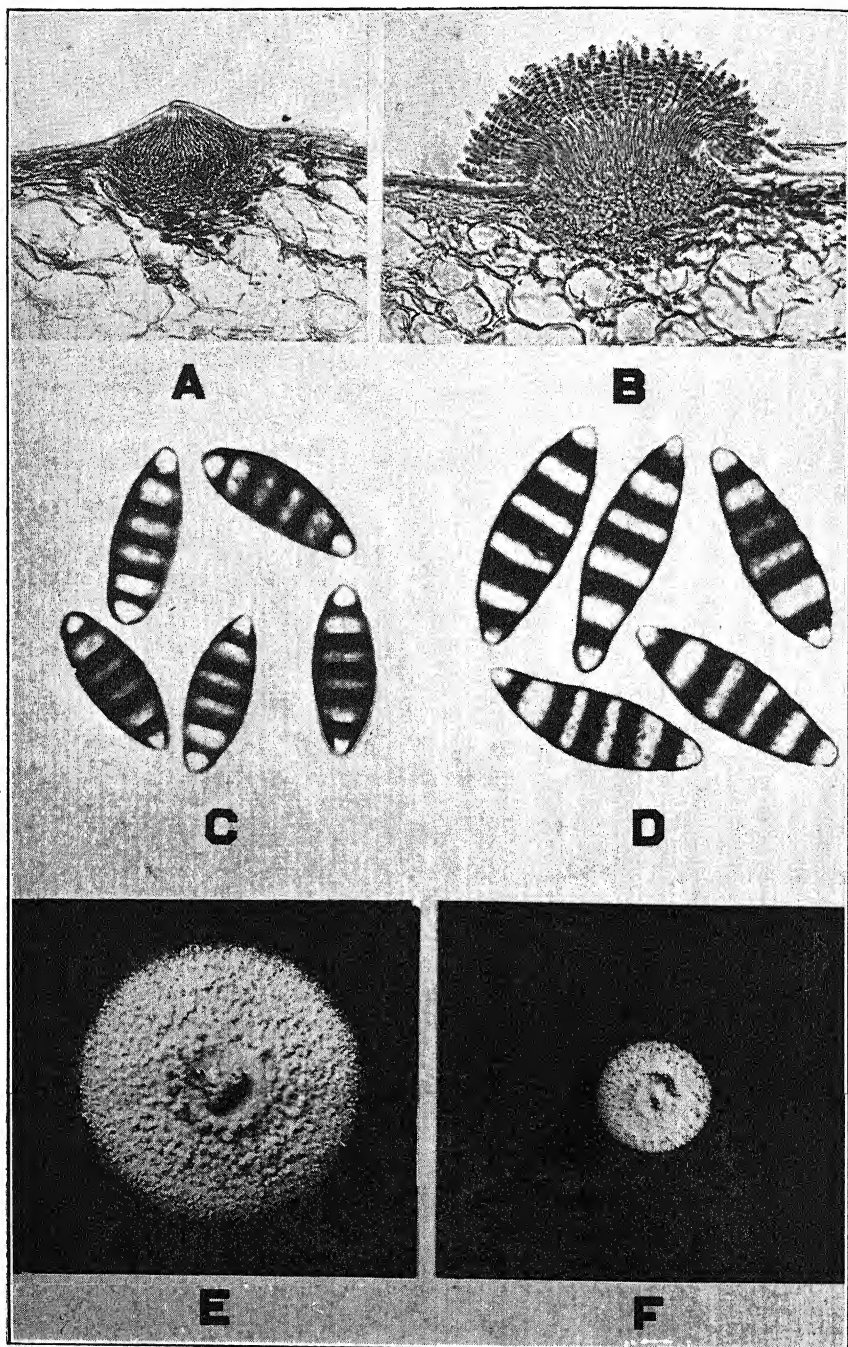


FIG. 2. A, B, D, F. *Coryneum berckmanii*. C, E. *C. cardinale*. A. Cross-section of an acervulus primordium. B. Cross-section of a mature acervulus. C, D. Comparison of spores of *C. cardinale* with those of *C. berckmanii*. E, F. Comparison of *C. cardinale* and *C. berckmanii* on lima-bean agar after growing 14 days at room temperature. All photomicrographs in this paper were made by the emulsion-resolution method described by McWhorter.⁸

Spore formation does not occur on any agar medium tested, unless the colony is subjected to temperatures below 65° F., and then only when the culture has aged for several months. When spores are produced they form in numerous pustules about 1 to 2 mm. in diameter. Abundant spore formation has been obtained in a few weeks by growing the fungus on wet, autoclaved oat grains. Spores formed only when the cultures were exposed to fluctuating, outdoor winter temperatures.

Pathological Studies

Berckman blight is difficult to establish artificially. Pieces of mycelium of *Coryneum berckmanii* inserted under the bark of small branches of *Thuja orientalis* failed to produce the disease. Branches bearing viable spores were hung on vigorous plants in a moist chamber; these plants did not become diseased. Trees sprayed with a spore suspension and placed in moist chambers remained free from signs of the disease until several months later when transplanted in outdoor plots. They then became infected, presumably as a result of the initial inoculation. Since it was observed that natural field infections occurred in the scale leaves near the tips of small branchlets careful inoculations were made in such scale leaves. Small bits of mycelium or spore masses were rubbed under the imbricated leaves. This produced a disease condition indistinguishable from natural infection. Acervuli and spores of *Coryneum berckmanii* developed on the inoculated branches, and the fungus was recovered in culture. Infection seemed to progress more readily and the secondary spread of the fungus seemed to become more extensive if the plants were exposed to the cold fall rains after the inoculations were made.

Under Northwest climatic conditions, primary infection occurs in October, or as soon as the fall rains begin. By November, infection centers 1 to 2 in. in diameter develop. Extensive spread of the fungus occurs at this time. Spores are formed on the new infections and these spores are washed or splashed to healthy foliage where they germinate and cause secondary infection. Thus the disease continues to develop until February or March. Until then, injury to the plant is evidenced only in the tissue actually invaded by the fungus. As the weather becomes warmer and drier, the spread of the fungus stops, but many of the small branches that have been girdled turn brown and die. Since the greater part of the injury becomes conspicuous at this time, one might conclude that the fungus is still developing. However, as mentioned previously, the mycelium has become inactive, thus making the spores the only means of reinfection and spread each fall. The disease is not systemic.

CONTROL INVESTIGATIONS

Investigations conducted during the past 2 years with various sprays for the control of Berckman blight, indicate that the disease can be satisfactorily controlled, even on severely blighted shrubs (Fig. 3). These results have been so promising that a report at this time is deemed advisable.

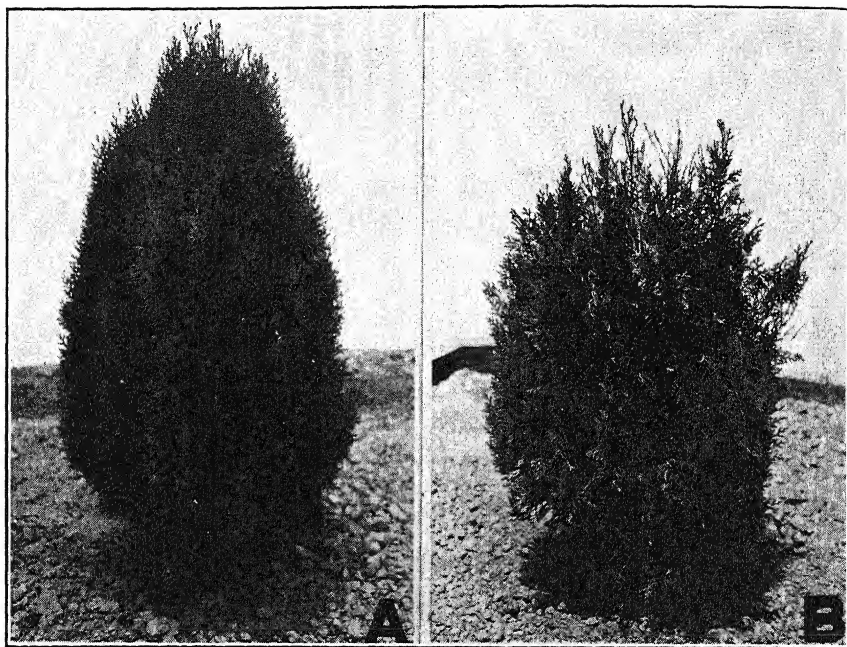


FIG. 3. Comparison of sprayed and nonsprayed trees. Both trees were severely infected at the beginning of the treatment. A. Tree protected by a copper spray. B. Tree left unsprayed for a check.

Since most nurserymen remove and burn plants as soon as they become diseased, it has been difficult to find large plantings of infected plants suitable for experimental work. Approximately 250 diseased plants have been planted in a nursery, near the Oregon Experiment Station, for studies on control of the disease and on its natural spread. This plot has been supplemented with experiments in nursery plantings whenever suitable diseased shrubs could be found. The majority of the plants used in control studies were severely blighted. Since the study of pathogenesis showed that infection began in the early fall, protective spray trials were initiated to circumvent this infection. For this reason all plants were given a thorough application of the trial sprays during the first week in September. A midwinter spray was anticipated, but a prolonged rainy and foggy season prevented the second application. Subsequent records proved that a second spray was unnecessary, and that one application of the proper spray is sufficient to control the disease. The sprays used in these tests were sold under the following trade names: red copper oxide, Rohm and Haas Cuprocide (96.5% cuprous oxide used in 1937, and Cuprocide 54, used in 1938); basic copper sulphate, Sherwin-Williams Basic-cop; copper ammonium silicate, California Spray and Chemical Corporation Coposil; copper oxychloride, Rohm and Haas Cupro-K; Sul Reso, Miller's Products Company, a sulphur-KOH-resin combination. Aresket 300 was used as a

spreader in 1937 and Vatsol was used instead of Aresket in 1938. The following discussion evaluates the sprays used.

Table 1 lists the sprays tested in 1937-38 and summarizes the results.

TABLE 1.—*Summary of 1937-38 spray tests for Berckman blight*

Treatment	No. of plants in treatment	No. of plants clean	No. of plants with trace of infection	No. of plants with scattered infection	No. of plants with heavy infection	Degree of infection	Spray injury
Red copper oxide 1-50	39	12	27	0	0	0.00	-----
Basic copper sulphate 2-50 ...	23	5	18	0	0	0.00	-----
Bordeaux 4-4-50	12	3	9	0	0	0.00	-----
Copper oxalate 1-50	4	4	0	0	0	0.00	-----
Copper phosphate 1-50	4	4	0	0	0	0.00	-----
Burgundy mixture	8	6	2	0	0	0.00	++++
Bordeaux 4-2-50	25	13	11	1	0	4.00	-----
Copper oxychloride 2-50	5	0	2	3	0	60.00	-----
Sulreso 1-30	8	0	2	2	4	75.00	++--
Sulphur dust	4	0	1	1	2	75.00	-----
Lime sulphur 1-40	4	1	0	1	2	75.00	+++-
Check	23	1	0	7	15	95.65	-----

* Trace of infection denotes that the disease was satisfactorily checked but one to three small branchlets had become infected without any secondary spread; scattered infection indicates three or more infection centers with some secondary spread; heavily infected indicates no control and often as much as 50 to 75 per cent of the foliage blighted. For comparison the degree of infection is calculated as percentage of plants with scattered and heavy infection. ++++ indicate very severe spray burn, +++- severe burn, ++-- very little burn, ----- no spray injury.

Most of the sprays caused very little injury, but certain ones injured the foliage. Alkaline Burgundy mixture, lime sulphur, and Sul-reso caused severe burn on some plants. The residue of Bordeaux 4-4-50 was objectionable on these ornamental plants, but Bordeaux 4-2-50 was less conspicuous. The other sprays did not discolor the foliage. The sulphur type sprays were not effective, but some of the copper sprays gave almost perfect control, even on plants that had 50 per cent or more of their foliage blighted the previous year. Red copper oxide, basic copper sulphate and Bordeaux mixture were used in several replications involving six different plantings. All 3 sprays gave remarkable control, considering the high potential source of infection from old blighted branches. The infection continued to progress in 22 of the 23 nonsprayed plants that served as checks. These checks evidenced a rapid rate of infection and subsequent increase in disease, a condition very different from that obtained in plants protected by successful sprays.

During the 1938-39 season, the experimental plot was supplemented by 3 commercial plantings that were severely blighted. Burgundy mixture and the sulphur sprays were omitted, and copper ammonium silicate was substituted for copper oxalate. Because of the limited number of plants, red copper oxide, basic copper sulphate and Bordeaux mixture were the only sprays used on the commercial plots. A summary of the results of

the 1938-39 spray tests is given in tables 2 and 3. Vatsol was used as a spreader at the rate of $\frac{1}{2}$ pound per 100 gallons of spray except where indicated.

TABLE 2.—*Summary of 1938-39 spray tests on experimental nursery*

Treatments ^a	No. of plants in treatment	No. of plants clean	No. of plants with trace of infection	No. of plants with scattered infection	No. of plants with heavy infection	Degree of infection
Copper ammonium silicate 6-100	11	10	1	0	0	0.00
Basic copper sulphate 2-50	11	9	2	0	0	0.00
Red copper oxide 3-100	11	9	2	0	0	0.00
Red copper oxide 3-100 ^b	10	6	3	1	0	10.00
Copper oxychloride 6-100	11	8	1	2	0	18.18
Copper phosphate 2-50	10	6	2	1	1	20.00
Bordeaux 4-4-50	25	8	9	6	2	32.00
Bordeaux 4-4-50 ^b	11	4	3	1	3	36.36
Check	19	0	0	0	19	100.00

^a Table headings have same interpretation as those of table 1.

^b Penetrol substituted for Vatsol as a spreader.

TABLE 3.—*Summary of 1938-39 spray tests on commercial nurseries*

Treatments ^a	No. of plants in treatment	No. of plants clean	No. of plants with trace of infection	No. of plants with scattered infection	No. of plants with heavy infection	Degree of infection
Red copper oxide 3-100						
Nursery A	56	34	22	0	0	0.00
Nursery B	131	49	72	10	0	7.63
Nursery C	81	74	7	0	0	0.00
Basic copper sulphate 2-50						
Nursery A	23	8	15	0	0	0.00
Nursery B	105	67	38	0	0	0.00
Nursery C	113	96	16	1	0	0.99
Bordeaux 4-4-50						
Nursery A	27	9	10	8	0	29.62
Nursery B	88	7	48	31	2	37.50
Check						
Nursery A	20	0	0	0	20	100.00
Nursery B	37	0	0	2	35	100.00
Nursery C	132	0	7	54	71	94.69

^a Table headings have same interpretation as those of table 1.

These data support the results of 1937-38, indicating conclusively that red copper oxide and basic copper sulphate will control Berckman blight. In the 4 different plantings where these 2 sprays were used 346 of the 531 plants were completely protected. Noticeable infection occurred in only 11 of the 174 plants in which the disease was not completely controlled.

Where incomplete control obtained, the amount of infection was very little when compared with the checks. 201 of the 208 plants left nonsprayed for checks were severely blighted by new infections.

Nursery B consisted of plants that were considered unsalable, many of them having only a few living branches. Therefore, this was an extremely severe test of the protective value of the sprays. Bordeaux mixture was consistently less effective in the control of the disease than was red copper oxide or basic copper sulphate. Thirty-three of the 88 plants sprayed with Bordeaux showed 5 to 10 scattered centers of infection where the fungus had become established.

Copper ammonium silicate also gave good control of the disease on the few plants sprayed, but considerable infection occurred on the plants sprayed with copper oxychloride and copper phosphate.

The use of Penetrol instead of Vatsol as a spreader decreased the efficiency of Bordeaux and red copper oxide.

These results seem to warrant the conclusion that Berckman blight can be controlled satisfactorily with one application of either red copper oxide or basic copper sulphate. A larger number of tests may prove that some of the other sprays are equally efficient, especially if uninfected plants or plants with only a slight degree of infection are to be protected.

There are several factors involved in an explanation of why this disease can be checked with one spray application even after the plant has become severely infected. The fungus is not systemic and depends on new infections each year for survival. The heavy colloidal particles of copper settle in the depressions formed by the scale leaves, and are very difficult to remove by rains. It is probable that the spores of the fungus would normally germinate and infect within these same depressions. Complete protection is maintained by applying the spray after the plants have become more or less dormant. Fortunately, they remain dormant until spring, which is also the end of the infection period of the fungus. It seemed probable that the disease would progress during the summer in home plantings, where owners syringed the plants at frequent intervals, thus making summer sprays necessary. No such development was observed, the probable explanation being that the summer temperatures prevented infection. This combination of features explains the unusual response of this disease to a single application of spray.

SUMMARY

An undescribed foliage disease of ornamental *Thuja orientalis* L. has caused serious losses in nurseries and home gardens of the Pacific Northwest.

The causal fungus is described as a new species, *Coryneum berckmanii*. It is distinct from other *Coryneum* species characterized by 5-septate spores, including *C. cardinale*, which is pathogenic on the Monterey cypress.

The fungus attacks several varieties of *Thuja orientalis* L. and also *Cupressus sempervirens* L., but has not been observed on other conifers growing in the immediate vicinity of diseased shrubs.

The disease can be satisfactorily controlled by one application of red copper oxide or basic copper sulphate provided the spray is applied before infections are initiated by early fall rains.

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ADDITIONAL FACTS REGARDING BACTERIOPHAGE LYTIC TO APLANOBACTER STEWARTI¹

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(Accepted for publication February 8, 1940)

INTRODUCTION

The bacteriophage has been recognized as a mysterious lytic agent whose origin has not yet been explained satisfactorily, and the rôle it plays has been only partially understood. We have come to think of the bacteriophage as an organic entity which causes a lysis of bacteria, or inhibits growth or probably functions as an important factor in bacterial dissociation. The fact that phages have been isolated so frequently from decaying organic matter, fecal material, soil, and river water has led to the assumption that they are widespread in nature. Yet, we are not always able to isolate a phage for a given bacterium from such sources.

The idea that the bacteriophage is evolved as a result of the antagonistic action of a host against an invading parasite has been suggested by several investigators. Wagner (10) found a lysin in healthy potato tubers and *Sempervivum hausmanii* following injection with cultures of *Bacillus vulgaris* and *B. asterosporus*. This lysin caused the membrane of bacteria to swell and dissolve and also inhibited the germination of spores.

Muncie and Patel (6) obtained a lytic principle for *Bacterium tumefaciens* from galls of sugar beet. Likewise, Chester (2) and Israily (4) have isolated bacteriophages effective against *Bact. tumefaciens* from galls induced by that organism. Both authors consider the appearance of the phage as an expression of resistance developed by the plants attacked.

The presence of lytic substances in plants was recognized before the bacteriophage was known. In 1897, Zinsser (11) observed that *Bacillus radicola* was not able to attack the aerial parts of the plant. He, therefore, advanced the hypothesis that these bacteria are killed by a bactericidal substance in the aerial tissue. A few years later Hiltner and Störmer (3) discovered that the juice of the roots of legumes, added to a bacterial colony, produced plasmolysis and disintegration of the bacteria. Also, Schiff-Giorgini (7) demonstrated, in tissues close to tumors produced by *B. oleae* upon olive branches, that there was a strong lytic and agglutinating action upon the organism causing the tumors.

Particular encouragement has been given to the investigation here reported by the facts that phage-treated cultures of pathogenic organisms

¹Approved for publication by the Director of the Ohio Agricultural Experiment Station.

no longer caused disease (6, 8), that such cultures were distinctly different from the original culture before the introduction of the phage (8), and also that from corn plants that had exhibited typical symptoms of Stewart's disease and, later, recovered, isolations of *Aplanobacter stewarti* with a phage associated with them were made (8).

EXPERIMENTAL PROCEDURE AND RESULTS

While investigating Stewart's disease in connection with field-corn hybrids, the author discovered that *Aplanobacter stewarti* could be isolated consistently from the margin of lesions, but not from the browned, middle portion. Water extracts prepared from the dead tissue of leaf lesions, tested against phage-free cultures of *Apl. stewarti*, showed that a lytic substance was present in nearly all of the samples taken (Table 1).

TABLE 1.—Effect of extracts of infected dead leaf tissue of corn leaves on *Apl. stewarti*

Field-corn hybrids	Culture No. 1	Culture No. 7
1. (4-8 × 02)	+ ^a	+
2. (1325 × Hy)	+	+
3. (4-8 × 84)	+	+
4. (Ill. 90 × 65)	+	+
5. (4-8 × 51)	+	+
6. (3028 × 56)	+	+
7. (51 × A)	+	+

^a+ signifies presence of a bacteriophage.

Water extracts were then prepared from the same hybrids, but this time from samples of healthy leaf tissue. The tissue was minced by grinding with a mortar and pestle, placed in flasks, covered with sterile, distilled water, and set aside overnight. The extracts were then filtered through a Chamberland-Pasteur candle, and graded portions of the filtrate were added to freshly prepared broth cultures of the test organisms. The same cultures were used in this experiment as in the previous one (Table 2).

TABLE 2.—Effect of extracts of noninoculated corn leaves on *Apl. stewarti*

Field-corn hybrids	Culture No. 1	Culture No. 7
1. (4-8 × 02)	+ ^a	—
2. (1325 × Hy)	— ^b	—
3. (4-8 × 84)	+	+
4. (Ill. 90 × 65)	—	+
5. (4-8 × 51)	+	+
6. (3028 × 56)	+	—
7. (51 × A)	+	+
8. (40B × 65)	—	—

^a+ signifies presence of bacteriophage.

^b— signifies absence of bacteriophage.

In the test with the extract of noninfected corn leaves, 2 samples did not contain a bacteriophage for either of the test cultures. Three con-

tained a phage for both cultures, and the remaining 3, for 1 culture only. Numerous similar trials always showed that a lytic substance could not be demonstrated in extract filtrates of noninfected corn leaves with as much regularity as in the extract of dead tissue from lesions.

From the examinations of extracts of both healthy and diseased corn-leaf tissue, an idea was gained that the lytic substance or bacteriophage was evolved as a result of the antagonistic action of the host against the invading parasite, and if such a reaction occurred naturally in the leaf after infection, it was thought probable that a similar reaction would occur in extract of macerated tissue if it were inoculated. In order to determine if such a reaction would take place in the laboratory, extracts of corn leaves were prepared in duplicate; one set was inoculated with *Aplanobacter stewartii* and the other was not inoculated.

After standing overnight, the filtrates of each respective set were tested against the culture of *Aplanobacter stewartii*, used for inoculating 1 set of extracts. In making such a determination, it was necessary to continue the cultures for at least 6 series, and in some cases for 10 or more, before the presence or absence of a lytic substance could be determined. Thus, for each extract examined, 3 tubes of nutrient broth were prepared, and all of them were inoculated with a few drops of broth culture of *Apl. stewartii* 18 to 24 hours old. One of the tubes of broth was held as a check, and to the other 2 tubes were added, respectively, 1 drop and 1 cu. cm. of filtrate. The cultures were allowed to incubate at 25° to 30° C. overnight. The bacterial growth, if any, was removed, by filtering, from the cultures to which the filtrates had been added previously. This filtrate was then used in a second series of broth cultures. The same routine was continued until the presence or absence of a lytic factor was established. This required at least 6 series of cultures for each extract.

The result of the inoculation of the corn leaf extract with *Aplanobacter stewartii* is evident in table 3. Extracts to which the corn wilt organism

TABLE 3.—The effect of inoculation of healthy corn leaf extract with *Apl. stewartii*

Field-corn hybrids	Not inoculated	Inoculated
1. (4-8 × 02)	+ ^a	+
2. (1325 × Hy)	- ^b	+
3. (4-8 × 84)	-	+
4. (Ill. 90 × 65)	-	+
5. (4-8 × 51)	-	+
6. (51 × A)	-	+
7. (40B × 65)	-	+
8. (02 × 1325)	-	+
9. (1325 × 02)	-	+
10. (56 × 40B)	+	+

^a + signifies presence of a bacteriophage.

^b - signifies failure to detect a bacteriophage.

had been added manifested the presence of a phage consistently, whereas the other duplicate set, with no inoculum present during the incubation,

did not. In all similar series of corn extracts investigated, lytic principles could be readily demonstrated if the test organism was added to the macerated tissue or extract for 12 to 24 hours before the examination was made. Filtrates of freshly prepared corn extract seldom contained a phage, whereas, after standing for a day, the lytic factor, if present, became filtrable in some cases, yet results were never consistent without previous inoculation of the extract. Filtrates of the test cultures of *Apl. stewarti* never showed the presence of a lytic factor.

All of the plant extracts so far considered were prepared from material obtained from the field or greenhouse. No attempt was made to sterilize them. Although washings of specimens with sterile water and extracts of soil usually failed to yield lytic substances, it was thought desirable to eliminate as many outside factors as possible. For this purpose a number of sweet corn plants of the Stowell's Evergreen variety were grown under sterile conditions in culture solution until they were about 6 inches high. At this time extracts were prepared from whole plants macerated under sterile, distilled water. These extracts were inoculated with a few drops from a culture of *Aplanobacter stewarti* and allowed to stand overnight. It was found that a lytic principle could be developed in the extracts of plants grown under sterile conditions, as well as of those from the field and greenhouse. This experiment shows that the lysin was inherent in the plant and not attributable to contamination.

All of the evidence obtained appeared to point to the plant as the source of the lytic factor, and it seemed reasonable to entertain the idea that the phenomenon under investigation might function as a mechanism of resistance in plants. If this be the case, other plants also should possess phage precursors. Accordingly, investigations were made, following the same method as with corn, of wheat, rye, oats, Kentucky bluegrass, foxtail grass, apple, celery, crimson clover, tomato, cabbage, stocks, hollyhock, raspberry, cockscomb, plum, peach, boxwood, cheat, and carrot. From all of these, lytic factors were isolated by using cultures of *Aplanobacter stewarti* known to be phage-free. No significant differences could be detected in the lytic principles obtained from these various sources, which would lead one to suspect that they were at variance with the so-called bacteriophage. All were transmissible in series, produced plaques upon solid media, and inhibited growth of the test culture by causing lysis. Attempts to develop a phage with extracts of peony leaves, roundleaf mallow, cutleaf birch, coleus and cherry were unsuccessful.

At this stage of the investigation attention was directed more particularly to the study of the nature of the lytic substance found in green plants. It seemed pertinent to learn whether there might be an analogy between the substance and the complement of fresh blood serum of animals. It was known that the bacteriophage of *Aplanobacter stewarti* is not inactivated when heated $\frac{1}{2}$ hr. at 65° C. In order to determine whether the lysin in plants is actually the phage or only a nonspecific precursor of it, the experiment shown in table 4 was made. Extract of wheat leaves was used.

TABLE 4.—*Thermo-inactivation study of the extract of wheat leaves*

1. Wheat extract not inoculated	+ ^a
2. Wheat extract not inoculated and filtrate heated at 56° C. for 30 min.	- ^b
3. Wheat extract inoculated	+
4. Wheat extract inoculated and filtrate heated to 56° C. for 30 min.	+
5. Wheat extract inoculated and filtrate heated to 60° C. for 30 min.	+
6. Wheat extract inoculated and filtrate heated to 65° C. for 30 min.	± ^c
7. Wheat extract heated at 56° C. for 30 minutes, then inoculated	-
8. Wheat extract heated at 45° C. for 30 minutes, then inoculated	+

^a + signifies presence of bacteriophage.

^b - signifies absence of bacteriophage.

^c ± lytic principle partially inactivated.

The results of this experiment show very definitely that a substance was present in the original wheat extract that was different from the bacterial lysis developed as a result of inoculation of the extract with a phage-free culture of *Aplanobacter stewartii*. The former was thermolabile at 56° C., whereas the latter was not; its activation temperature was above 65° C. When this test was applied to corn and tomato leaf extracts, the same results were obtained. It was likewise found that after extracts of horse feces, and of corn, wheat, oats, and rye grains had been heated at 56° C. a lytic principle could not be developed (9). Yet, when a phage-free culture was brought into association with nonheated water extracts of such substances, a lytic principle was formed, specific for the culture introduced and more consistently filtrable. In addition to this the temperature of inactivation was raised.

From the facts presented, it became evident that many isolates from diseased plants may have a phage associated with them. In nutrient broth medium a bacterial culture is very seldom killed during the period of lysis. The secondary or resistant growth, which later develops, will continue to reproduce itself, either in liquid or on solid medium, just as the phage-free culture will do. The lytic principle can be demonstrated any time by adding the filtrate to a phage-free culture. In testing a series of isolates for pathogenicity, one may find some of them strongly virulent and others slightly or not at all so.

Development of a bacteriophage for *Aplanobacter stewartii* in macerated plant tissue could not be accomplished with all isolates of that organism. This was suspected to be because of the presence of a lytic principle in some of the cultures. Tests made in this connection with a culture known to contain a strong lytic principle yielded negative results, i.e., another lytic principle could not be developed for a culture that already contained one.

The problem of separating a bacterium from a bacteriophage or of inactivating the lytic principle in a culture has offered particular difficulty. Other investigators have reported some degree of success by adding anti-bacteriophage serum to a culture containing the lytic factor (1). Best results were obtained when the phage had not acted upon the culture for a very long period. Such a separation undoubtedly occurs in nature, possibly in a number of different ways. A method was devised that may approximate natural conditions to some extent.

The secondary growth, following lysis, of a susceptible culture of *Apl. stewarti*, which had been growing in nutrient broth at a reaction of pH 6.3, was cultured in the same medium, but the reaction was changed with malic acid to pH 3.85 to pH 4.00. Transfers were made to fresh tubes of broth of the same reaction 5 times at 24-hour intervals. Each time only a very small drop was carried over. Dense clouding of the medium occurred when the acid was added, and after the solution had stood for a time, a heavy sediment separated. The supernatant liquid remained clear, presenting no evidence of growth; yet, when transfer was again made to the original broth, normal, homogeneous clouding developed, as in the parent culture before treatment with the phage. The growth recovered from the acid broth was found to be susceptible to lysis by the same lytic principle as the parent culture; its filtrate no longer contained a phage, and a lytic factor could again be developed for it in rye-leaf extract. These 3 criteria were considered sufficient evidence that the culture containing the phage had been rendered phage-free by the acid-broth treatment. Adequate data have not yet been accumulated to determine whether the lytic principle was actually inactivated by the acid broth, or, being unable to increase, was eliminated by dilution. This method has been followed 3 times with cultures that have been in association with the phage 2 weeks and 3 months, respectively. In both cases these cultures have been rendered again phage-susceptible or, in other words, free of the lytic principle.

In the course of this investigation it was surprising to find that so many of our cultures of *Aplanobacter stewarti* were phage-free. Since a majority of the isolates were separated out with the use of a highly oxidizing medium, commonly designated as Ivanoff's medium (5), it was considered probable that this medium might be unfavorable to phage regeneration. Accordingly, streak transplants from a culture known to contain the phage were made upon Ivanoff's medium and allowed to develop for 10 days. Some of the bacterial growth, selected from regions as far removed from the original streak as possible, was returned to nutrient broth. The recovered culture was susceptible to lysis by the phage that it originally contained, whereas cultures similarly grown upon potato-dextrose agar still contained phage originally associated with them, and were not susceptible to lytic action. Ten cultures of *Aplanobacter stewarti*, which had been found refractory to phage action, were selected for further trial of this method. The growth that was recovered in each instance was found to be phage-free and, therefore, susceptible to lysis.

Phage-free isolates could be obtained readily with potato-dextrose or nutrient agar medium from diseased seedlings of Golden Bantam and Golden Gem varieties of sweet corn. In the seedling stage these varieties possess very little resistance to *Aplanobacter stewarti*. In this work, the corn wilt organism only has been used. A few attempts were made to remove lytic factors from cultures of *Bacillus amylovorus* and *Bacterium tumefaciens*. These species apparently do not remain viable in broth as strongly acid as *Apl. stewarti* can withstand. It will be necessary to determine the critical acid range for each species, if the acid-broth method proves to have general application.

A group of 6 cultures of *Aplanobacter stewarti* was selected for the purpose of phage generation in extract of rye leaves. These cultures had been obtained from specimens collected at widely separated locations in Ohio. Some were isolated from sweet corn and some from hybrid field corn. There were marked variations in rate and intensity of growth. A phage was developed for each of the cultures by using portions from the same lot of rye extract. After 3 passages in series, each phage was tested against the other 5 cultures. The responses of the different cultures to each respective phage are represented in table 5.

TABLE 5.—Response of different isolates of *Apl. stewarti* to lytic principles developed from rye, for each respective isolate^a

Cultures	Bacteriophage					
	1	2	3	6	7	10
1	++++	—	—	+++	++	+++
2	—	+	+++	++++	++	—
3	++++	++	++++	++++	+	++
6	++	++++	++++	++++	++++	+
7	+	++++	++++	++++	++++	+
10	+++	++	—	+++	+++	++++

^a++++ signifies complete lysis after 24 hours.

— indicates no visible effect whatsoever. Gradations between these two extremes are represented by +, ++, and +++.

Some of the phages were weak, some were strong. The one developed with No. 2 culture was weak for its own culture but strong for Nos. 6 and 7. In some cases lysis was scarcely noticeable after 6 hours but complete after 24. In others it was complete after 4 hours. Cultures represented by Nos. 1 and 10 were known to be phage-free. No investigation was made of the others in this connection. Probably No. 2 culture already contained a weak phage acquired from the corn plant from which that culture was isolated. This study shows that when a common precursor is used, such as occurs in rye-leaf extract, the phages produced vary with the cultures. This method may be of value in determining identity or diversity of different isolates.

If the development of bacteriophages in plant extracts can be associated with resistance, it should follow that sweet-corn varieties susceptible

to Stewart's disease do not have them, or if phage precursors are present, they must be very weak. An examination was, therefore, made of 9 different varieties of sweet corn, some of which were generally recognized as wilt-resistant and some not. The plants were grown in the greenhouse. After attaining a height of 4 to 6 inches, the tissue was macerated in sterile water, inoculated with a few drops of *Aplanobacter stewarti*, and set aside until the following day. Filtrates from these extracts were then added to broth cultures of the test organism (Table 6). The presence of the

TABLE 6.—Tests for presence or absence of phage precursors in extracts from susceptible and resistant sweet-corn varieties

1.	Golden Bantam	— ^a
2.	Whipples White	+ ^b
3.	Golden Sunshine	—
4.	Stowell's Evergreen	+
5.	Spanish Gold	+
6.	Golden Colonial	+
7.	Golden Evergreen	—
8.	Country Gentleman	+
9.	Early White Market	—

^a — indicates absence of bacteriophage.

^b + indicates presence of bacteriophage.

lytic principle in extract of resistant varieties was manifested in the 2nd and 3rd series of cultures, whereas, with the susceptible strains, there was no indication of a lysin in the 10th series. Later, tests of Golden Bantam plants that had reached the tasseling and silking stage, showed that a precursor was present at that time. This is in accord with field observations that seedling plants of that variety are very susceptible to Stewart's disease, but that older plants approaching maturity exhibit marked resistance. A similar examination was made of field corn, particular attention being given to self-pollinated lines and single crosses. In the pure lines there was considerable variation among different plants of the same line. With some a strong lytic principle could be developed, yet not in others. A lytic factor can be developed from Golden Cross Bantam sweet corn at all stages of growth.

DISCUSSION

The phage precursor in plants remains active after the death and decomposition of the plant and thus continues to maintain a biological balance between host and parasite. It has been found still active for *Aplanobacter stewarti* in tomato leaves that had been dried at temperatures below 45° C. until they were brittle. Undoubtedly, there are wide variations among phage precursors of different plants and, even of plants of the same species. Probably all corn plants contain them, the extremely susceptible, as well as the most resistant to bacterial wilt. The writer has never examined a corn plant that had succumbed to the wilt that did not contain a bacteriophage. In some plants the mechanism of resistance is quickly activated; in others its action is delayed. Apparently, in some

cases, such a long period is required for effective lytic activity to be built up that the plant dies before infection can be checked. A corn plant with a strongly active precursor quickly stops the advance of infection, in many instances before the brown or necrotic stage has developed. Seedling plants of Golden Bantam and Stowell's Evergreen sweet-corn varieties are typical examples of the two extremes.

Certain environmental factors may influence or control the formation of phage precursors. They are always present in the seed and leaves of foxtail grass grown out of doors; yet, when the same species are grown under glass, no bacterial lysins can be demonstrated. Such erratic behavior never has been observed in corn or other plants investigated. It was noted in some instances that when plant extracts did not give rise to lytic principles, there was an agglutinating effect upon the culture. This effect was not transmissible in series and was, therefore, lost by dilution. The phenomenon of lysis probably is only one of several different manifestations of the antagonistic action of a plant against the introduction of a foreign body.

To what extent other plants and their respective bacterial parasites follow the same pattern as corn and *Aplanobacter stewarti* remains yet to be determined. A lytic factor, transmissible in series, has been prepared for *Bacterium tumefaciens* from rye-leaf extract. It seems reasonable, nevertheless, that every plant possesses a mechanism of resistance, either physical or chemical, or both, by means of which it maintains its individuality and combats foreign invaders. The rapidity and intensity with which the mechanism of resistance can be brought into action vary greatly in different species and in individual plants of the same species. This conforms to common observations of varying degrees of susceptibility and resistance.

The presence of a phage developed as the result of the antagonistic action of the plant against the parasite may, in many cases, account for the reduced virulence, or loss of virulence, of the bacteria present. Cultures containing a phage may not be recognized as having any relation to the real pathogen and, for that reason, may be discarded as saprophytes. It is suggested that an analytical investigation of some of our so-called saprophytic organisms might produce some very interesting results.

SUMMARY

A nonspecific phage precursor inactivated by heating at 56° C. for 30 minutes, has been found in many plants. When this substance comes in contact with susceptible bacteria, a reaction occurs, resulting in the formation of a transmissible lytic principle that is not inactivated at 60° C. and only partly at 65° C. This is believed to be the origin of the bacteriophage in plants and to function as a mechanism of resistance.

These lytic principles vary with differences in cultures of bacteria used to produce them.

In corn varieties, susceptible to bacterial wilt, the phage precursor was lacking or very weak, whereas, in resistant varieties, it was strong.

Several methods have been found effective in rendering cultures of *Aplanobacter stewarti* free of the bacteriophage.

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MYCELIAL EXTENT BEYOND BLISTER RUST CANKERS ON *PINUS MONTICOLA*¹

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INTRODUCTION

The longitudinal enlargement and development of the lesion or "canker," resulting in western white pine (*Pinus monticola* Douglas) from infection by *Cronartium ribicola* Fischer, naturally depends on the growth of the mycelium: as the mycelium advances into new susceptible tissue, so the cankered area enlarges. Colley (2, page 625) stated that in northern white pine (*P. strobus* L.) "the advancing tips of the invading hyphae . . . generally extend a little beyond the line," i.e., the edge of the "etiolated area" or limit of

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surface discoloration; but he reported no numerical data on number of cankers examined, actual extent, or variation in extent. Subsequent workers (9, 5, 6, 1, 4) have assumed that surface discoloration approximates or bears a fairly constant relationship to mycelial extent in both northern and western white pine. Martin, Gravatt, and Posey (8, page 7) found that pruning cuts in northern white pine made $1\frac{1}{4}$ in. or more back of the visible edge of the canker, *i.e.*, proximad of the proximal edge of the canker, remained healthy, indicating that mycelial extent usually does not exceed this distance, although it is possible that exposure of tissue at the cut might have killed any hyphal tips present at or slightly beyond this point. Lachmund and Hansbrough (7) found that, when cankered branches of western white pine were pruned about $\frac{1}{2}$ in. above the lower limit of discoloration, *i.e.*, distad of the proximal limit of discoloration, a large majority of the cankers continued to grow proximally for a time, indicating that mycelium probably extends beyond the limit of discoloration.

The purpose of this study was to explore a method for determining the magnitude and constancy of mycelial extent beyond the limit of surface discoloration. If this distance were found to be reasonably constant, confidence in previous work, using bark discoloration as an indication of the growth of the fungus, would be strengthened. On the other hand, if this distance were found to be variable, attempts would be made to correlate the mycelial extent with certain readily determinable canker characters that might plausibly influence this extent. If mycelial extent were found in this preliminary study to be closely correlated with one or more of such characters, additional data might eventually provide a reliable means for field estimation of mycelial extent by consideration of these characters. Ability to make such estimates would aid in determining the expected amount of forest damage by enabling more accurate diagnosis of killing cankers, and in its reduction by intelligent pruning in infected, valuable, managed stands and in ornamental individuals.

No previous work of this character with *Cronartium ribicola* has been discovered.

MATERIALS AND METHODS

Cankered stems and branches in all stages of infection were collected in October, 1935, at 3 stations (Crystal Creek near Fernwood, and East Fork of the St. Maries River and Gold Center near Clarkia, Shoshone County, Idaho), from western white pine growing on reasonably comparable sites, all bordering on stream types and thus assuring similar moisture relations.

At the time of collection, individual canker data were recorded on field data sheets for the first 10 items (see below), the distal and proximal limits of surface-bark discoloration for each canker being marked with delicate knife incisions (Fig. 1) and the outside branch diameters at these points measured by calipers. Where trunk cankers were too large to preserve conveniently, the distal and proximal outside diameters were measured; and the bark, with possibly an annual ring or two of xylem, was stripped from

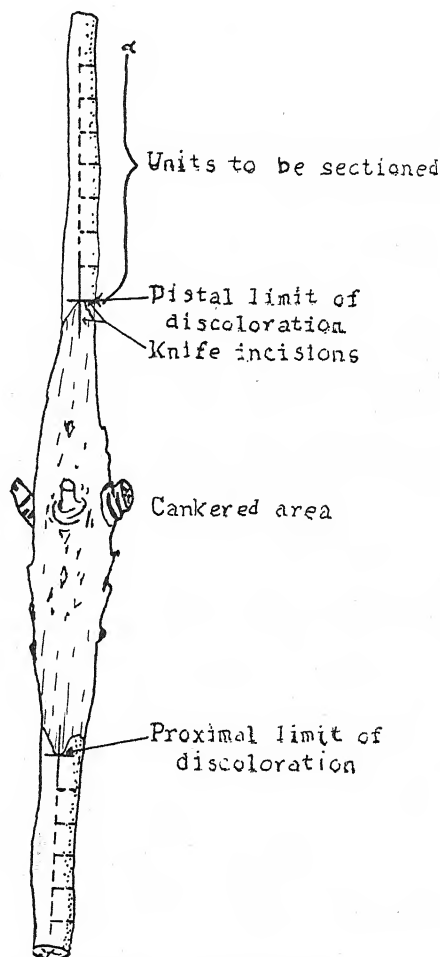


FIG. 1. Diagram of canker, showing knife incisions marking limits of discoloration and units to be sectioned.

the wood and preserved in formalin acetic alcohol. All materials collected were classified as to age and stage of infection according to Lachmund's scheme (5, page 684). Data for items 11 to 14, listed below, were recorded in the laboratory, as the information was obtained.

Basic Data Recorded for Each Canker

1. Age of tree.
2. Height of tree.
3. Year growth infected.
4. Length of canker (distance between distal and proximal limits of discoloration).
5. Distal diameter of cankered internode (at limit of surface discoloration).

6. Center diameter of cankered internode.
7. Proximal diameter of cankered internode (at limit of surface discoloration).
8. Stage of canker.
9. Discolored portion of cankered branch circumference (at widest point).
10. Nondiscolored portion of cankered branch circumference (at widest point).
11. Bark thickness at:
 - a. Distal limit of discoloration;
 - b. Proximal limit of discoloration;
 - c. Distal limit of mycelium;
 - d. Proximal limit of mycelium.
12. Extent of mycelium beyond distal limit of discoloration.
13. Extent of mycelium beyond proximal limit of discoloration.
14. Remarks.

From the incision marking the extremity of surface discoloration, the bark was removed in units 0.5 cm. long. Each unit was sectioned radially, using a hand microtome. Sections were mounted in acid fuchsin in Amann's medium and examined microscopically for mycelium. This procedure was followed for all units, each unit being consecutively farther from the canker, until a section was found through which the mycelium did not completely extend. Following this unit, two more units were examined as a check on the presence of random hyphae. When the distal or proximal limits of the mycelium were definitely determined, the total extent was recorded in centimeters and/or hundredths.

RESULTS

Mycelial Extent

When the above listed data on mycelial extent (items 12 and 13) were arrayed, the mycelium in every canker sectioned was found to extend beyond the limits of surface discoloration and to vary considerably, the distances ranging between 0.49 and 3.16 (one 5.56) cm. with mean and standard error of 1.68 ± 0.06 cm. distally, and between 0.46 and 3.24 cm. with mean and standard error of 1.63 ± 0.05 cm. proximally, or a mean of 1.66 for all 262 measurements. Thus no significant difference was found between distal and proximal means or extremes. Colley's statement for northern white pine (1) may thus be extended to western white pine. But these results indicate that the assumption of others, that surface discoloration bears a fairly constant relationship to mycelial extent, is hardly justified for western white pine.

The extent means and ranges for each canker collection group, for all groups, and for groups lumped by position of canker and condition of stem or branch are shown in table 2. Inspection of the data discloses that the differences between the means in any lumped grouping and between corresponding distal and proximal means, although statistically significant, are

of no practical significance (with the exception of the 2 flagged groups). This means that, so far as these meager data for 3 areas in a single locality can be relied on, differences in geographic location and the direction of growth from the center of a canker do not influence mycelial extent. Differences of practical significance appear between the lumped groupings, however, mycelial extent being greatest for cankers on green stems,⁴ less for those on green branches, and least for cankers on flagged branches. This means that position of canker and condition of stem or branch must be taken into account in estimating mycelial extent.

Correlations: Grouped Data

Since the ranges are wide, the extent measurements for each lumped grouping and for all cankers, with a few omissions, were separated successively into several classes under each of the 4 following characters, and the coefficients of correlation calculated: estimated age of canker; stage of canker; thickness of bark at outer limits of surface discoloration; and diameter of cankered internode at outer limits of surface discoloration.

Age of Canker. The extent measurements for cankers on green stems (Table 1 Groups I-II) and green branches, Groups III-IV-V, were separated first on the basis of estimated age of canker, following Lachmund (5), into 1-year classes and correlated with age as the independent variable. Cankers on flagged branches were ignored because of the greater uncertainty of estimating their age. The coefficients of correlation (r) were calculated by the method of Thurstone (10) and found (Table 2) to be moderate for the cankers on green stems (.59 distally and .67 proximally) and low for the cankers on green branches (.25 distally and .27 proximally). The statistical significance of differences between paired distal and proximal values was not calculated but they are assumed to be unimportant. The probability that these coefficients could arise by random sampling from an uncorrelated population was determined by the method of Fisher (3); the resulting values of P indicate that this probability is slight, being equivalent to odds of better than 99 to 1 in 3 cases and slightly less than 99 to 1 in one case that it could not arise by chance. Thus the calculated coefficients of correlation, although not high, are all judged to be highly significant.

Stage of Canker. The same extent measurements were separated next on the basis of stage of canker, again following Lachmund (5), the actual stage of the canker providing the class interval. The same statistical procedures were followed as for age of canker. The results (Table 2) are comparable with those for age of canker, except for proximal growth in the cankers on green stems, where the coefficient of correlation is .20 lower than the comparable value for age of canker and are, accordingly, less significant.

Thickness of Bark at Outer Limits of Surface Discoloration. The same extent measurements, plus those for cankers on flagged branches in Group

⁴ Greater mycelial extent in green stems than in green branches might be expected, since Lachmund (6) showed that the fungus grows faster in green stems than in green

TABLE 1.—Extent of mycelium beyond limits of surface discoloration, for all cankers studied, grouped by collection group and station, position of canker, and condition of stem or branch

Group and station	Collection station	Position of cankers	Condition of stem or branch	Trees sampled	Direction from center of canker							
					Distal				Proximal			
					Cankers studied	Mean extent	Standard error	Range	Cankers studied	Mean extent	Standard error	Range
I	Crystal Creek	Stem	Green	No. 11	No. 11	Cm. 2.46	± 0.33	Cm. 1.47-3.16 (one 5.56)	No. 11	Cm. 2.24	± 0.14	Cm. 1.51-3.22
II	Gold Center	Stem	Green	12	12	2.27	± 0.06	1.97-2.61	11	2.30	± 0.04	2.01-2.51
II		Stem	Green	23	23	2.36	± 0.16	1.47-3.16 (one 5.56)	22	2.27	± 0.07	1.51-3.22
II	Gold Center	Branch	Green	8	43	1.61	± 0.07	0.90-3.01	43	1.69	± 0.11	1.01-3.24
IV	E. Fk. St. Maries	Branch	Green	3	11	1.23	± 0.19	0.49-2.21	11	1.29	± 0.20	0.51-2.24
V	Crystal Creek	Branch	Green	6	39	1.47	± 0.09	0.66-2.78	40	1.57	± 0.13	0.69-2.83
-V		Branch	Green	17	93	1.51	± 0.05	0.49-3.01	94	1.59	± 0.09	0.51-3.24
IV	E. Fk. St. Maries	Branch	Flagged	5	25	1.13	± 0.11	0.46-2.30
V	Crystal Creek	Branch	Flagged	3	5	1.93	± 0.37	1.55-2.41
-V		Branch	Flagged	8	30	1.26	± 0.11	0.46-2.41
				43 ^a	116	1.68	± 0.06	0.49-3.16 (one 5.56)	146	1.63	± 0.05	0.46-3.24

^a The total number of trees is less than the sum of the group totals because certain trees in groups IV and V bore both green and flagged branch

IV, were separated next on the basis of thickness of bark at the outer limits of surface discoloration into 0.2-mm. classes. The same statistical procedures were followed as with age and stage of cankers. The results (Table 2) are somewhat erratic, coefficients of correlation varying from .28 for distal growth in the cankers on green branches (Groups III-IV-V), to .80 for proximal growth in the cankers on flagged branches (Group IV), probabilities of significance being high for all coefficients save the one for distal growth in the cankers on green stems (Groups I-II). Differences between paired distal and proximal values are assumed again to be unimportant.

Upon examination of the scatter diagram representing the lowest correlation (Groups III-IV-V, distal) it was noticed that most of the deviation is in cankers on bark under 1.0 mm. in thickness. The extent measurements for such cankers in this group were accordingly thrown out and the remaining measurements recalculated. The coefficients of correlation (Table 2) were thereby raised by .32 and .34. This means that, in the cankers studied, mycelial extent is moderately to highly correlated with thickness of bark when thickness is 1.0 mm. or greater.

Diameter of Cankered Internode at Outer Limits of Surface Discoloration. The same extent measurements first correlated with thickness of bark were separated finally on the basis of diameter of the cankered internodes at the outer limits of the surface discoloration, into 0.5-cm. classes. Again, the same statistical procedures were followed. The results (Table 2) are somewhat erratic but comparable with those for thickness of bark, coefficients of correlation ranging from .40 for distal growth in the cankers on green branches (Groups III-IV-V) to .80 for proximal growth in the cankers on flagged branches (Group IV), and probabilities of significance being high for all coefficients. Differences between paired distal and proximal values are again assumed to be unimportant. This means that in the cankers studied mycelial extent is moderately to highly correlated with diameter.

The extent measurements for all cankers (nongrouped by position of canker or condition of stem or branch) also were correlated with each of the same characters. The results (bottom of Table 2), as anticipated, show values of r intermediate between the values for the different lumped groupings under each character and emphasize the influence of position of canker and condition of stem or branch upon mycelial extent. The results bear out the inconsistency and probable unimportance of differences between paired distal and proximal measurements.

CONCLUSIONS

The following tentative conclusions regarding factors relating to mycelial extent, in this limited number of cankers from 3 ecologically comparable areas in a single locality at a single time of year, seem justified:

1. In this locality, geographic location does not influence mycelial extent.
2. Direction of growth (whether distal or proximal) from the center of a canker does not influence mycelial extent.

TABLE 2.—Coefficients of correlation between extent of mycelium beyond limits of discoloration and certain readily determinable characters

Canker group	Position of cankers	Condition of stem or branch	Direction from center	Cankers studied	Characters (independent variables)			
					Age of canker	Stage of canker	Thickness of bark	Diameter of internode
				No.	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
I-II	Stem	Green	Distal Proximal	21 21	2-10 years .59 < .01 .67 < .01	First sympt.— <i>fr. several</i> .57 < .01 .47 < .05-.02	1.0-2.9 mm. .49 sl > .02 .70 < .01	1.00-6.90 cm. .66 < .01 .61 < .01
					2-12 years .25 sl > .01 .27 < .01	First sympt.— <i>fr. several</i> .26 < .01 .26 < .01	0.3-3.0 mm. .28 < .01 .30 < .01	0.30-1.20 cm. .40 < .01 .45 < .01
III-IV-V	Branch	Green	Distal Proximal	51 56	1.0-2.9 mm. ^b .60 < .01 .64 < .01
					0.5-3.1 mm. .80 < .01	0.35-2.34 cm. .80 < .01
IV V	Branch Branch	Flagged Flagged	Proximal Proximal	25 5
					No. 114 .39 < .01 114 .28 < .01	No. 114 .30 < .01 114 .41 < .01	No. 114 .48 < .01 144 .48 < .01	No. 114 .42 < .01 144 .49 < .01
All	Distal Proximal	var. var.				

^a *P* indicates the significance attaching to the calculated value of *r*, by measuring the probability that the calculated value of *r* could not have arisen by chance; the lower the value of *P*, the higher the probability. Values of *P* under .01 represent odds of over 99 to 1; values over .1 represent odds of under 9 to 1. The values of *P* were secured by entering Fisher's (3) Table V.A. with 2 less than the number of pairs in the sample.

^b Since most of the deviation was in cankers on bark under 1.0 mm. in thickness, such cankers were thrown out in this recalculation.

3. Position of cankers (whether on stem or branch) seems to influence mycelial extent as well as the relation of other canker characters to mycelial extent, which is greater in stem cankers than in branch cankers.

4. Condition of stem or branch (whether green or flagged) seems to influence mycelial extent as well as the relation of other canker characters to mycelial extent.

5. Increasing age of canker (at least to 12 years) seems to be related to increasing mycelial extent, especially in stem cankers. Since this character can not be determined accurately, especially for cankers on flagged branches, and is only slightly related in branch cankers, it can probably be ignored in estimating mycelial extent.

6. Stage of canker seems to be similar to age of canker in these respects.

7. Increasing thickness of bark at outer limits of surface discoloration, especially when 1.0 mm. or greater, seems to be related to increasing mycelial extent. If it were accurately determinable in the field, bark thickness would provide a useful factor in estimating mycelial extent.

8. Increasing diameter of cankered internode at outer limits of surface discoloration seems to be related to increasing mycelial extent. Since this character can be readily and accurately determined in the field, it should provide a useful factor for field estimation of mycelial extent.

9. Further investigation should include cankers from other localities, other sites, and flagged stems, as well as green stems and branches and flagged branches at different times of year, and could be restricted to correlations of internode diameter with mycelial extent.

SUMMARY

Microscopic measurements of longitudinal extent of mycelium of *Cronartium ribicola* beyond the outer limits of surface discoloration in the bark of *Pinus monticola* were made on the distal ends of 116 cankers in green stems and branches and on the proximal ends of 146 cankers in green stems and green and flagged branches from 3 ecologically comparable areas in a single locality. An attempt was made to determine the relationship of mycelial extent to certain readily determinable characters.

Mycelial extent was found to extend in all measurements beyond the outer limits of surface discoloration and to vary considerably, ranging from under 0.5 cm. to over 3.0 cm. (over 5.5 cm. in one) with a mean of 1.66 cm. No practical mean difference was found between proximal and distal measurements or, for each type of canker, between measurements for the different areas. Mean extents were found to be approximately 2.3 cm. for cankers on green stems, 1.5 to 1.6 cm. for cankers on green branches, and 1.2 cm. for cankers on flagged branches.

Mycelial extent was found to be variously correlated with estimated age of canker, stage of canker, thickness of bark at outer limits of surface discoloration, and diameter of cankered internode at outer limits of surface discoloration. As foreshadowed by the extent means, the coefficients of cor-

relation were found to be more uniform for each position of canker and condition of stem or branch than for each of the independent variables. Internode diameter was judged to be a useful factor for field estimation of mycelial extent. Its evaluation for different positions of canker and conditions of stem or branch must await the accumulation of additional measurements from other localities, other sites, and flagged stems, as well as green stems and branches and flagged branches at different times of year.

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PHYTOPATHOLOGICAL NOTES

Powdery Mildew of Lespedeza.—Powdery mildew has been observed on various strains of annual lespedeza in the nursery at Arlington Experiment Farm, Arlington, Virginia, each year since 1935. While the disease usually has developed too late in the season to cause serious losses, it does appear to cause a certain degree of premature defoliation. Perithecia of the mildew develop in October each year and the fungus has been identified as *Microsphaera diffusa* Cooke and Peck. In the fall of 1938 an epidemic of this powdery mildew developed on seedlings of several strains of annual lespedeza in the greenhouse. The abundant development of mildew perithecia on the leaves of these strains is illustrated in figure 1. Notes on the relative susceptibility of these strains of lespedeza to powdery mildew are presented in table 1. It would appear from these data that strains of *Lespedeza striata* (Thunb.) H. and A. are more susceptible than are strains of *L. stipulacea* Maxim. This agrees with field observations at Arlington Farm, Virginia, where Kobe lespedeza appears each year to be more heavily mildewed than Korean. No powdery mildew developed on two perennial species of lespedeza (*L. sericea* (Thunb.) Benth. F. C. No. 04730 and *L.*

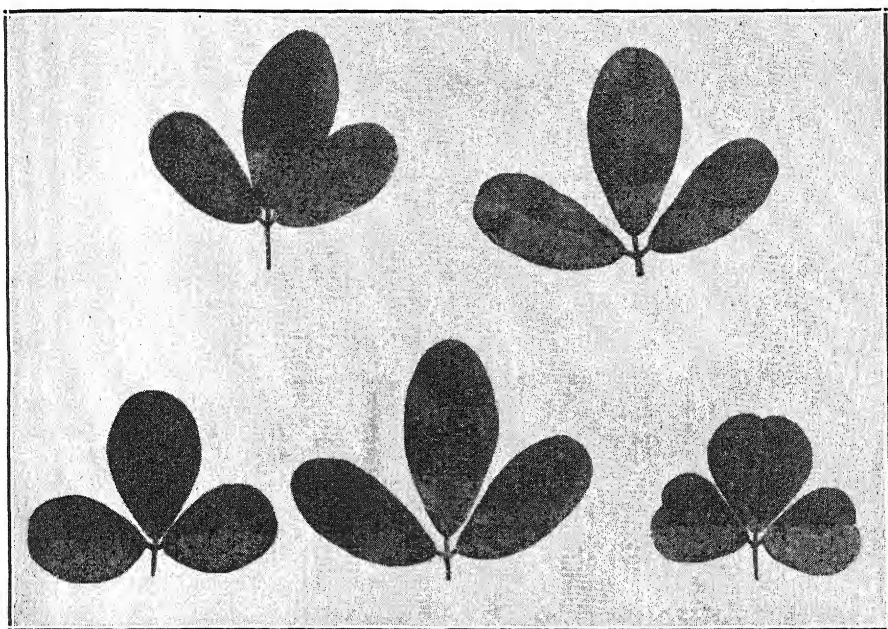


FIG. 1. Perithecia of *Microsphaera diffusa* on the leaves of various strains of annual lespedeza. $\times 1\frac{1}{2}$. Above: Common F.C. No. 22590 and Common F.P.I. No. 81742; below: Late Korean F.C. No. 19601, Kobe F.C. No. 22456 and Standard Korean F.C. No. 22457. Greenhouse material, Arlington Farm, Virginia, October 17, 1938.

sp. F.P.I. No. 82098, a species closely related to *L. sericea* and *L. juncea* Wall.) growing on the same greenhouse bench with the annual lespedezas, nor has the disease been observed in the extensive field plantings of perennial lespedeza at Arlington Farm. Salmon,¹ however, lists 3 perennial

TABLE 1.—Relative susceptibility of various strains of annual lespedeza to powdery mildew (*Microsphaera diffusa*) in the greenhouse at Arlington Farm, Virginia, in 1938

Species	Common name	F.C. or F.P.I. No.	Severity of infection ^a		
			Series 1	Series 2	Series 3
<i>Lespedeza stipulacea</i>	Early Korean	19604	2
	Standard “	22457	2	2	3
	Late “	19601	1	3—	2
<i>Lespedeza striata</i>	Common	81742	4	4	4
	“	22590	3	4—	4—
	Kobe	22456	3	4	4—

species of lespedeza (*L. capitata* Michx., *L. hirta* (L.) Hornem. and *L. violacea* (L.) Pers.) as hosts of this powdery mildew, so its appearance upon cultivated perennial species of this genus probably should be expected.—H. W. JOHNSON, C. L. LEFEBVRE, and T. T. AYERS, Bureau of Plant Industry, U. S. Department of Agriculture, Arlington, Virginia.

^a In the scale used to denote severity of infection 1 indicates very light, 2 light, 3 moderate and 4 severe infection.

¹ Salmon, E. S. A monograph of the Erysiphaceae. Mem. Torr. Bot. Club 9: 1-292.

*Delayed Reduction of the Diploid Nucleus in Promycelia of Ustilago zaeae.*¹—In the course of studies on the genetics of *Ustilago zaeae* (Beckm.) Ung., the author obtained certain results interpretable genetically only on the basis of the first meiotic division being delayed to at least the second or third division of the diploid nucleus of the chlamydospore. Insofar as known, no proof that this occurs in the smuts has been published previously, although Christensen² reported solopathogenic or diploid lines in *U. zaeae* in which the reductional divisions occurred in the next chlamydospore generation.

It was reported previously³ that the chlamydospores produced by crosses between certain haploid lines of *Ustilago zaeae* germinated very abnormally, a high percentage of the promycelia disintegrating without producing sporidia. Among the viable sporidia solopathogenic lines occurred.

In the present work, one or more single sporidia were isolated from 158 chlamydospores produced by these abnormal crosses. Young corn plants were inoculated repeatedly with the cultures obtained alone and in compatible combinations, and it was found that both haploid and diploid sporidia (based on whether or not segregation for sex or compatibility factors had taken place) were produced on the promycelia of 3 chlamydospores. Figure 1 shows the arrangement of the haploid and diploid sporidia on the promycelia of

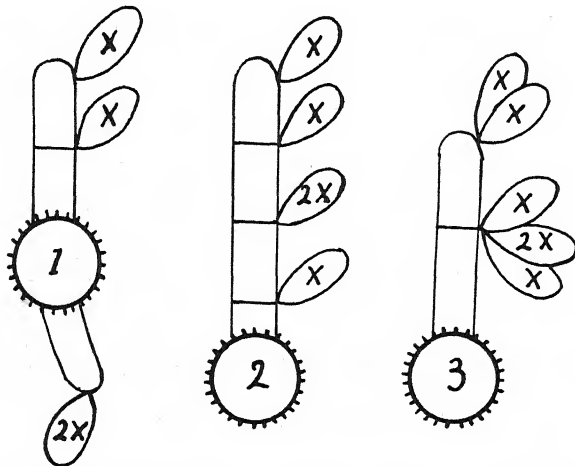


FIG. 1. The arrangement of haploid and diploid sporidia on the promycelia of chlamydospores of *Ustilago zaeae*.

idia as they were produced on the promycelia. It may be seen from this figure that in chlamydospore 1 the first meiotic division was postponed to

¹ Paper No. 1786 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

² Christensen, J. J. Studies on the genetics of *Ustilago zaeae*. *Phytopath. Zeitschr.* 4: 129-188. 1931.

³ Chilton, S. J. P. The occurrence of lysis in certain crosses of *Ustilago zaeae*. (Abst.) *Phytopath.* 28: 5. 1938.

at least the second division of the diploid nucleus and in chlamydospores 2 and 3 to at least the third division.

No generalization is possible because of the abnormal germination of the chlamydospores; yet the above results indicate that delayed reductional divisions may occur in certain cases.—ST. JOHN P. CHILTON, U. S. Regional Pasture Research Laboratory, Bureau of Plant Industry, State College, Pa.

A Method of Inducing Spore Production by Cercospora apii Fres. in Pure Culture.—The *Cercosporae* as a group have been found to produce few typical conidia in pure culture. Nagel,¹ by the use of a transfer technique, was able to keep many species in a sporulating condition. Others have noted sporulation of other species under special conditions. Klotz² reported that only on celery refuse were spores of *Cercospora apii* produced in any abundance. Following this lead, the author³ has found a simple method of inducing conidial formation by *C. apii*.

To an Erlenmeyer flask soil was added to a depth of 2 cm. Muck, compost, and sand have all been used with little difference in results. Water was added, so that after the whole had been sterilized there was a little free water in the soil but none on the surface. After sterilization of the soil, celery leaflets were placed in contact with it. These were then sterilized by autoclaving for 20 min. Mycelium transferred to the celery leaflets grew readily, and conidia developed in 6 days at room temperature. They continued to be produced near the outer edge of the growth until the celery leaflets were covered by the fungus. The conidia were as typical as those found in the field. The conidiophores, however, were not typical. For the most part, the distances between geniculations were exceedingly long, hundreds of microns, as compared with those found in the field, 10–30 microns. Fewer conidia were produced by growing the fungus on sterilized leaves alone in test tubes containing $\frac{1}{2}$ in. of water. The leaf was loosely crumpled in the tube.

In estimating the number of conidia produced the flattened end of a needle about 0.5 mm. wide was drawn across 1 cm. of the surface of the culture where conidia were developing. The conidia collected were deposited in a drop of water on a slide and examined under low power of the microscope. The conidia were considered as abundant when a hundred or more could be found in one field.

Old, as well as newly isolated, cultures produced an abundance of conidia. One culture, isolated in 1936 and carried on agar till 1939, produced an abundance of spores when grown as described above. Cultures that had lost their characteristic color on agar did not produce spores.—RALPH W. LEWIS, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

¹ Nagel, C. M. Conidial production in species of *Cercospora* in pure culture. *Phytopath.* 24: 1101–1110. 1934.

² Klotz, L. J. A study of the early blight fungus, *Cercospora apii* Fres. *Mich. Agr. Exp. Stat. Tech. Bull.* 63. 1923.

³ Lewis, R. W. Studies of the life history of *Cercospora apii* Fres. Unpublished master's thesis. Mich. State College. 1937.

Galls on Pseudotsuga macrocarpa induced by Bacterium pseudotsugae.

—An aerial gall on Douglas fir, *Pseudotsuga taxifolia* Britt., has been described by Hansen and Smith¹ as caused by *Bacterium pseudotsugae* Hansen and R. E. Smith. This organism does not resemble the crown-gall organism, *Bacterium tumefaciens* Smith and Townsend, and the writer has not succeeded in producing crown galls on *P. taxifolia* or *P. macrocarpa* Mayr.

Inoculations on the big-cone spruce, *Pseudotsuga macrocarpa*, with a culture of *Bacterium pseudotsugae* furnished by H. N. Hansen gave galls (Fig. 1, A, B) that resembled those described by Hansen and Smith on

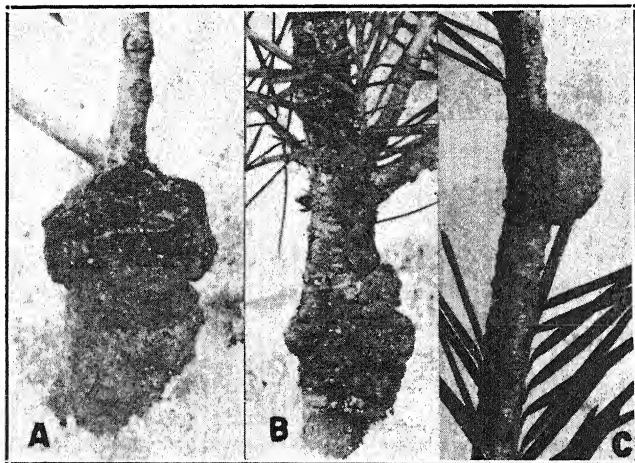


FIG. 1. Inoculation with *Bacterium pseudotsugae*. Photographed after 30 months: A and B. Galls on *Pseudotsuga macrocarpa*. C. Gall on *P. taxifolia* var. *glauca*.

Pseudotsuga taxifolia. Inoculations made at the same time as those on *P. macrocarpa* developed galls (Fig. 1, C) on *P. taxifolia* var. *glauca*, the Douglas fir of Colorado. *Abies concolor* was inoculated but no galls were produced.—CLAYTON O. SMITH, Division of Plant Pathology, University of California Citrus Experiment Station, Riverside, California.

¹ Hansen, H. N., and Ralph E. Smith. A bacterial gall disease of the Douglas fir. *Science* 77: 628. 1933. *Hilgardia* 10: 569-577. 1937.

COMPARATIVE WILT INDUCTION BY *ERWINIA TRACHEIPHILA* AND *PHYTOMONAS STEWARTI*

HUBERT A. HARRIS¹

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INTRODUCTION

The causal factors for wilting in plants induced by different species of bacteria and fungi remain doubtful, in most instances, notwithstanding numerous researches. To determine these factors, 3 chief methods have been employed in earlier investigations of the problem. The commonest is to induce wilting of either plants or cuttings with the filtrates of the various nutrient solutions in which the organism has been cultured. Another method, according to Melhus, Muncie, and Ho (17), and LeClerc and Durrell (14), is to make fluometric determinations of wilt-infected stems or roots. A more recent method, Linford (15), Yoshii, and Masano (28), and Ahmet (1), but one that has received only a limited consideration, is a study of the transpiration of the wilting plants. Usually, only one of these methods has been employed in a single investigation and no attempt has been made to combine all three methods.

The present investigation has attempted to obtain toxicity, transpiration, and fluometric data on 2 bacterial wilts; that caused by *Erwinia tracheiphila* (E. F. S.) Holland in cucumber (*Cucumis sativus* L.), and the wilt of sweet corn (*Zea mays* L. var. *saccharata* Bailey) produced by *Phytophthora stewarti* (E. F. S.) Com. S. A. B.

REVIEW OF LITERATURE

An extensive resumé of the literature on the wilting of plants induced by bacteria and fungi is not included in this paper, inasmuch as Rosen (20), White (27), Haymaker (10), Hursh (11, 12), Schaffnit and Lüdtke (21), Ahmet (1), Grossman (9), and Brown (4) have adequately reviewed the more important investigations.

The mechanical-plugging theory was cited by Stewart (24) in 1897 and by Smith (23) in 1899. This theory suggests that wilting is occasioned by an interference with the water supply by the growth of the organism within the vessels, and by the formation of gums, resins, and tyloses so frequently induced by the invasion of a wilt organism. The theory was based on morphological observations. Sections of wilt-infected plants frequently showed many of the vessels filled with an abundant growth of the organism and the plant was assumed to have wilted because of an interference with the water conduction.

¹ The writer is gratefully indebted to Professor C. F. Hottes for the facilities of the Physiology Department and for suggesting the wick culture technique. Appreciation is expressed to Professor N. E. Stevens for his interest in the problem and for suggestions in the preparation of the manuscript. Thanks are due Dr. A. G. Vestal and Dr. Charlotte L. Grant for advice in maintaining a constant soil-moisture range.

Melhus, Muncie, and Ho (17) devised an apparatus, the fluometer, to measure the rate of water flow through stems and roots. They determined that there was a considerable reduction in the passage of water through cabbage stems infected with *Fusarium conglomerans* Woll. and alfalfa stems infected with *Phytomonas insidiosum* (McCulloch) Bergey *et al.* It was concluded that the wilting resulted from a partial or complete plugging of the water ducts. LeClerc and Durrell (14) also made fluometric determinations on alfalfa infected with *Phyt. insidiosum*. They attributed the immediate cause of wilting to vascular plugging.

A chief criticism of the mechanical-plugging theory is that wilting may occur in plants showing sparse mycelial growth in the vessels. Bearing upon this criticism is the gas-emboli theory of Tochinai (26). In physiological studies of *Fusarium lini* Bolley, *in vitro*, the organism showed ready gas production from decomposition of carbohydrates. He concluded that the rapid wilting of flax seedlings was occasioned by an obstruction of the xylem tubes by gas emboli arising from carbohydrate decomposition. The gas emboli, thus, could be produced by a trifling mycelial development in the lumina.

Van der Meer (16) studied the factors influencing the slime disease of tobacco, tomato, and other crops in Sumatra caused by *Phytomonas solanacearum* E. F. S. He concluded that wilting of tobacco and tomato resulted from water deficiency and that this physiological drought was caused by the plugging action of gums secreted by the organism in the vascular system. Ahmet (1) studied tracheomycoses produced by *Fusarium vasinfectum* Atk. in cotton and *F. lycopersici* Sacc. in tomato. He concluded that the primary cause of wilting, in both instances, was mechanical and that toxins were of secondary importance.

The toxic filtrate theory of wilting has received much more prominence in recent years than has that of mechanical plugging. The method usually used in experiments interpreted as supporting this theory is to culture the organism in nutrient solution and then to insert cuttings or intact plants in the filtrates. If wilting results, it is concluded that the pathogen has secreted wilt-producing toxic filtrates. Hutchinson (13) presented the toxin theory of wilting in 1913 when he grew *Phytomonas solanacearum* in bouillon culture. An alcoholic precipitate of the culture solution, when dissolved in water and injected into healthy tobacco plants, produced wilting. He concluded that wilting resulted chiefly from interference with the osmotic pressure consequent on protoplasmic intoxication and that the water conduction, too, was interfered with by the formation of gum masses.

The transpiration effects in relation to wilting also have been considered by some investigators. Gilman (7) attributed the yellowing of cabbage by *Fusarium conglomerans* to a slow drain by the fungus on the water supply, and this, together with a high temperature, caused an increased growth of the fungus and an increased transpiration of the host. Tisdale (25) mentioned the same possibility as one of the probable causes of flax wilt.

Linford (15) studied the transpiration histories of 12 healthy pea plants and 12 infected with *F. orthoceras* App. and Woll. var. *psi* Linf. Five infected plants showed an upward trend of the transpiration rate coincident with wilting; 3, a questionable upward trend; and 2, a reduction. He suggested that wilting might have resulted, not from a diminished water supply, but rather from an excess loss of water from the leaves. Toxic filtrates from the pathogen were believed to be transported to the leaves and to cause an alteration in leaf protoplasts so as to lead to loss of their normal powers of water retention and, subsequently, to their loss of turgor and to the wilting of the leaf. Yoshii and Masano (28) observed the transpiration of excised soy beans inserted into the filtrate of *F. niveum* E. F. S. cultured in Czapek's solution. The decrease of the transpiration of the wilting plants was parallel to the progress of the wilt phenomenon. Four plants were used and all showed a decrease in the transpiration rate during wilting.

PROCEDURE

Filtrate Toxicity Determinations

Both organisms were cultured in 500 cc. of a broth medium, comprising Difco bacto-beef extract 3 g., Difco bacto-peptone 5 g., to 1000 cc. of distilled water in one-liter Erlenmeyer flasks. The solutions were adjusted to an initial pH of 6.8 and sterilized in the Arnold steam sterilizer for 1 hour on three successive days. After growth of the organism in the medium for 3 weeks at 24° to 26° C., the solutions were filtered twice through No. 1 "Whatman" filter paper prior to filtration through a Berkefeld "N" filter. Distilled water then was added to restore the solutions to the original volume. The filtrates were adjusted to the pH of the original solutions before the toxicity tests were made and before a portion was autoclaved at a pressure of 15 lb. for 20 minutes. The filtrates (20 cc.) were placed in test tubes and plant cuttings, whose stems were cut under water, were inserted. Readings were taken on the wilt tests after 20 hours. The plants tested were: (Purdue Agr. Exp. Sta. Hybrid No. 1421) and cantaloupe (Fordhook Famous).

Transpiration Determinations

Soil Series. In attempting to determine the comparative transpiration of wilt-infected and noninfected plants grown in soil, the method described by Grant (8), a modification of the method used by Veihmeyer and Hendrickson,² was followed. The inner surface of No. 2 tin cans, together with cover lids punched with half-inch holes, were paraffined and each container was tared to an equal weight by the addition of glass beads. An equal weight of air-dry, brown, silt loam (525 g.), screened through a $\frac{1}{16}$ in. wire mesh screen, was placed in each can. Uniformity of packing was accomplished by dropping each container 5 times through a distance of 3

² This method has not been published. Access to it was obtained through the courtesy of Dr. A. G. Vestal in a letter from Veihmeyer entitled: "Direct method of determining wilting point."

inches. Enough water was added to each soil-filled can to bring the soil moisture to the "optimum" content cited by Grant (8), who lists the upper limit (oven-dry weight) for this range as 35.15 per cent and the lower as 29.72 per cent. The containers then were planted to the respective seeds, and the plants were rotated, 1 revolution per $3\frac{1}{2}$ min., on greenhouse turntables, $3\frac{1}{2}$ ft. in diameter, to eliminate inequality of lighting and of other environmental factors. The inoculations were made in the stems by needle punctures with the bacterial-wilt organism, cultured on beef-extract agar. The stems of the control plants were punctured similarly, but no inoculum was introduced. Transpiration readings were made by daily weighings and the addition of water from a burette to restore the soil moisture content to the upper limit. Two containers were not planted to serve as checks on the evaporation loss of moisture from the soil. The transpiration losses were corrected for the mean daily water loss from these two containers.

Nutrient-solution Series. The wick-culture method of Raines (18) was employed for the transpiration studies of plants cultivated in nutrient solutions. Stone jars (650 cc. capacity), $5\frac{1}{2}$ in. high, were fitted with small 50-cc. glass dishes, attached to one-inch width strips of galvanized tin by means of DeKhotinsky's cement. In the studies of *Erwinia tracheiphila*, blotting paper was used on the inside of the containers. However, the growth of cellulose-destroying organisms made the use of this material unsatisfactory. Subsequently, in the studies of *Phytomonas stewarti*, glass cloth, as recommended by Raines (19), was substituted. The glass cloth was impregnated with a thin hydrochloric acid-sodium silicate gel. The gel solution consisted of equal volumes of hydrochloric acid solution (sp. gr. 1.10) and sodium silicate solution (sp. gr. 1.09).

The R5C2 nutrient solution of Shive (22) was used. The containers were tared to equal weight by the addition of glass beads. 100 cc. of the nutrient solution was added to the bottom of each container, and 40 cc. to the glass dish in the top. Daily weighings were made, as in the case of the soil series, and the nutrient solution was replaced in the glass dish from a burette. Also, 2 nonplanted containers served as checks on the evaporation loss and as corrections for the daily transpiration readings. During the course of the experiments, the nutrient solution in each container was changed every 3 days.

Fluometric Determinations

For determining the comparative rate of water flow through noninfected and wilt-infected stems, the fluometer devised and described by Melhus, Muncie, and Ho (17) was assembled. All stems were cut under water prior to insertion in the fluometer.

EXPERIMENTAL RESULTS

Erwinia tracheiphila

Toxicity Effects of Filtrates. The toxicity of the filtrates from *Erwinia tracheiphila* was determined by culturing the organism in beef-extract-

broth solution. Both the autoclaved and nonautoclaved filtrates were tested on cuttings of both cucumber and sweet corn. The former plants were 4 to 7 in. tall and in the 5- to 8-leaf vegetative stage while 3 of the latter were 8-10 in. tall and in the 3-leaf stage. The wilt-inducing tests of the different solutions were made at temperatures ranging from 22° to 26° C. The tests were performed in duplicate, and the results are shown in table 1.

TABLE 1.—*Effect of filtrates from Erwinia tracheiphila upon cucumber and sweet corn*

Medium	Trial number	Initial pH	pH after three weeks	Color ^c	Plants tested		Plants wilted		Plants recovering	
					Ca	Sb	C	S	C	S
Beef-extract broth filtrate	1		8.2	Raw Sienna	10	10	10	8	5	6
	2		8.0	do	10	10	10	10	8	7
Beef-extract broth filtrate (autoclaved)	1		8.2	do	10	10	10	9	4	7
	2		8.0	do	10	10	10	8	6	4
Beef-extract broth (uninoculated)...	1	6.8	6.8	Yellow Ocher	10	10	1	0	0	
	2	do	do	do	10	10	0	0		
Tap water	1				10	10	0	0		
	2				10	10	0	0		

a—cucumber.

b—sweet corn.

c Ridgway's color standards.

It will be seen that the filtrates of *Erwinia tracheiphila* induced wilting of cuttings of both cucumber and sweet corn. The autoclaved portions of the filtrates effected wilting as readily as the nonautoclaved. Wilting of the cuttings was not occasioned in the noninoculated beef-extract broth nor in tap water, and the single cucumber cutting which wilted, during the first trial in the uninoculated solution, is regarded as not significant. The filtrates caused a greater degree of wilting of cucumber than those of sweet corn. Also, a few more cuttings of the latter recovered in tap water than did those of cucumber.

Transpiration Effects

Soil Series. The cucumber plants, grown in soil and employed for the transpiration studies with *Erwinia tracheiphila*, were 5 to 6 inches tall and in the 4-6-leaf stage when inoculated. The temperatures ranged from 15° to 24° C. during the experiment. Additional lighting was provided from 5 p.m. to 8 a.m. daily by 12 150-watt bulbs above the turntables.

The comparative mean daily transpiration losses of the infected and noninfected plants in the soil series are shown in figure 1, A. From the 3rd

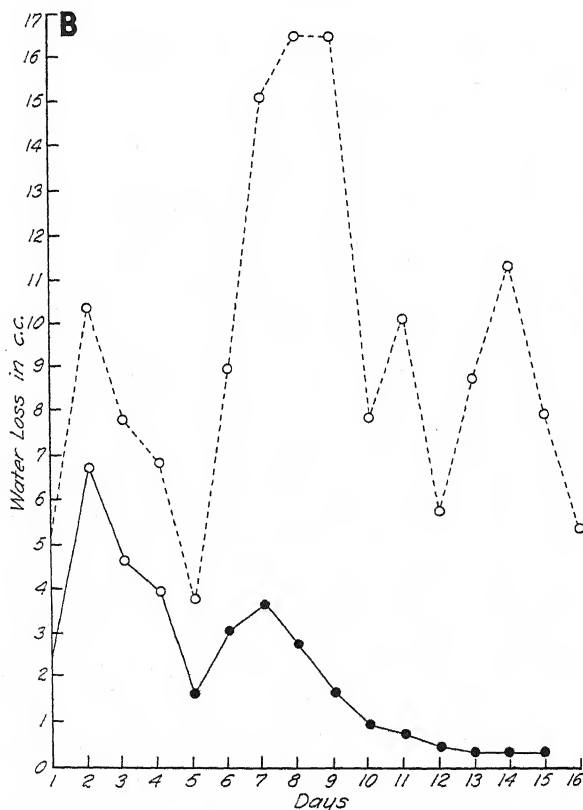
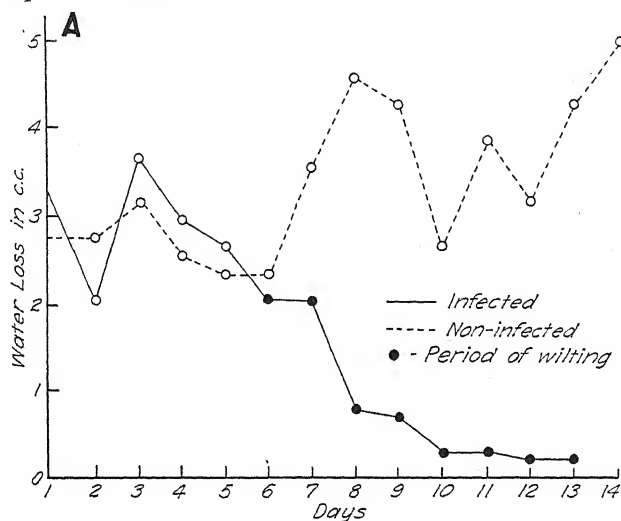


FIG. 1. Mean daily transpiration loss from cucumber. A. Twenty plants infected with *Erwinia tracheiphila* and 30 plants noninfected in soil series. B. Eleven plants infected with *E. tracheiphila* and 15 plants noninfected in nutrient solution series.

to the 5th day, the mean transpiration rate for the infected plants is consistently greater than that for the noninfected. However, on the 6th day, which preceded the period of initial wilting for the infected plants, the mean transpiration loss falls below that of the noninfected plants. The rate for the latter remains constant as for the fifth day. Then, as the symptoms of wilt are increasingly manifest, a progressive decrease occurs for the infected plants, whereas an increase results for the noninfected plants.

Nutrient Solution Series

The series of plants grown in nutrient solution were 4 to 6 inches tall when inoculated, and in the 3- to 5-leaf stage. No additional lighting was provided for these plants; and the summer temperatures during the experiment ranged from 20° to 40° C. The comparative mean daily transpiration losses from the plants in the nutrient solution series are shown in figure 1, B. The transpiration rate for the infected plants, does not deviate markedly from the latter until the 6th and 7th days after inoculation. Thereafter, the general progressive decrease characteristically accompanies the advanced stages of wilting.

Fluometric Determinations

The comparative fluometric determinations for the water flow through stems of cucumber infected with *Erwinia tracheiphila* and those noninfected are shown in table 2. The 10 noninfected stem sections permit a mean flow

TABLE 2.—Comparative fluometric determination of water flow in cc. for 10-minute intervals through 5 cm. stem lengths of cucumber infected with *Erwinia tracheiphila* and those noninfected

Noninfected			Infected		
Plant number	Diameter of stem mm.	cc.	Plant number	Diameter of stem mm.	cc.
1	4.3	2.2	11	5.0	.3
2	5.0	3.3	12	5.2	.1
3	4.8	2.3	13	4.0	.4
4	4.5	2.1	14	5.0	.6
5	5.0	3.4	15	4.0	.2
6	5.0	2.8	16	5.2	.7
7	4.8	2.5	17	4.0	.2
8	4.2	1.9	18	4.8	1.1
9	4.4	1.6	19	5.2	.6
10	4.5	2.0	20	5.0	.2
Total	46.5	24.1	Total	47.4	4.4
Mean	4.65	2.41	Mean	4.74	.44

of only .44 cc. However, the mean diameter of the noninfected stems is 4.65 mm. as compared with 4.74 mm. for the infected stems. Proportionately computed on that basis, the noninfected stems would permit a mean flow of 2.46 cc. Calculated on this same basis the water flow through the

infected stems is reduced comparatively by 82.13 per cent. These conclusions would not be affected by more exact quantitative data involving measurement of vessel diameters instead of those of the stems.

Phytomonas stewarti

Toxicity Effects of Its Filtrates. The toxicity of the filtrates of *Phytomonas stewarti* cultured in beef-broth solution was tested on cuttings of both sweet corn and cucumber. The incubation period and temperature of the cultures and the stage of development of the cuttings, as well as the temperature range during the tests for wilting, are the same as stated for like tests of *Erwinia tracheiphila*. The results from duplicate trials are shown in table 3. The data are similar to those results obtained with the

TABLE 3.—Effect of filtrates from *Phytomonas stewarti* upon sweet corn and cucumber

Medium	Trial number	Initial pH	pH after three weeks	Color ^c	Plants tested		Plants wilted		Plants recovering	
					S ^a	C ^b	S	C	S	C
Beef-extract broth filtrate	1		8.4	Mars Yellow	10	10	9	10	9	3
	2		8.2	do	10	10	8	10	8	6
Beef-extract broth filtrate (autoclaved)	1		8.4	do	10	10	10	10	8	4
	2		8.2	do	10	10	8	10	7	4
Beef-extract broth (uninoculated) ..	1	6.8	6.8	Yellow Ocher	10	10	0	1		0
	2	do	do	do	10	10	0	0		
Tap water	1				10	10	0	0		
	2				10	10	0	0		

^a—sweet corn.

^b—cucumber.

^c—Ridgway's color standards.

filtrates of *E. tracheiphila*. Cuttings of both sweet corn and cucumber were wilted in both the autoclaved and nonautoclaved filtrates of the beef-extract broth, while wilting was not affected in the noninoculated solution nor in tap water. It would appear, too, that sweet corn is more tolerant to the toxic effects of the filtrates than cucumber.

Transpiration Effects

Soil Series. The plants of sweet corn, grown in soil and used for the transpiration studies with *Phytomonas stewarti*, were 6 to 9 in. in height and in the 3- to 4-leaf stage when inoculated. The temperatures varied from 18° to 24° C. during the experiment. Additional daily lighting was supplied the plants.

The mean daily transpiration losses of the plants in the soil series are represented in figure 2, A. This graph shows that, during the first 8 days,

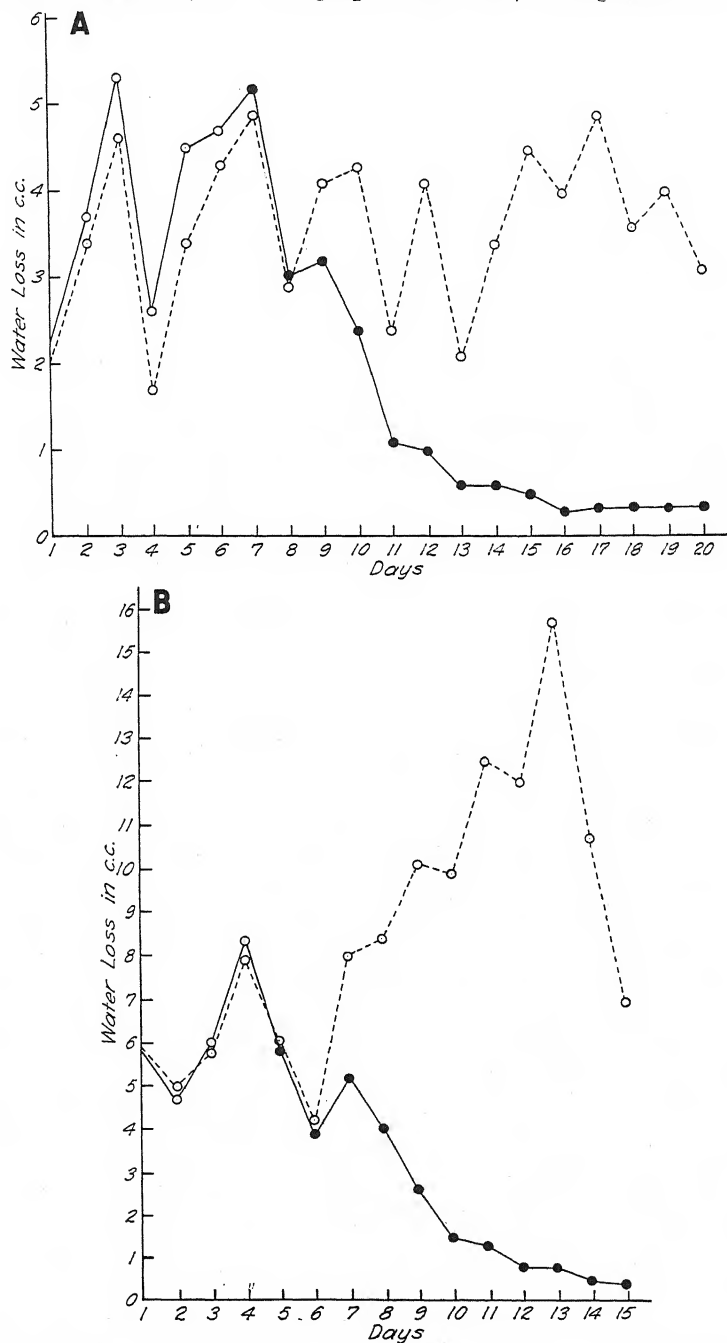


FIG. 2. Mean daily transpiration loss from sweet corn. A. Seventeen plants infected with *Aplanobacter stewarti* and 30 plants noninfected in soil series. B. Twenty plants infected with *A. stewarti* and 20 plants noninfected in nutrient solution series.

the mean transpiration losses for the infected plants are greater than those for the noninfected plants. However, on the 9th day after inoculation, the losses for the infected plants fall abruptly below those for the noninfected plants and continue to decrease progressively as wilting occurs.

Nutrient Solution Series

The plants of sweet corn grown in nutrient solution for the transpiration studies with *Phytomonas stewarti* were 5 to 8 inches tall and in the 3-leaf vegetative stage when inoculated. The temperatures ranged from 20° to 24° C. during the experiment, and additional lighting also was supplied the plants.

The comparative mean daily transpiration losses for the plants in the nutrient solution series are shown in figure 2, B. During the first 6 days, the comparative difference between the mean transpiration rate for the infected and noninfected plants is slight. On the 7th day after inoculation, however, the transpiration losses for the infected plants show a decided decrease as compared with the losses for the noninfected plants. Subsequently, the general progressive decrease, which characteristically accompanies the advancement of wilting, is noted.

Fluometric Determinations

The comparative fluometric results obtained with stem sections of sweet corn infected with *Phytomonas stewarti* and those noninfected are shown in table 4. The ten noninfected stems have a mean diameter of 3.45 mm.

TABLE 4.—Comparative fluometric determination of water flow in cc. for 10-minute intervals through 4 cm. stem lengths of sweet corn infected with *Phytomonas stewarti* and those noninfected

Noninfected			Infected		
Plant number	Diameter of stem mm.	cc.	Plant number	Diameter of stem mm.	cc.
1	3.5	.8	11	3.0	.2
2	2.5	.6	12	3.0	.1
3	4.5	1.1	13	2.5	.1
4	3.5	.9	14	3.5	.3
5	3.5	.7	15	4.0	.3
6	3.0	.8	16	3.5	.2
7	2.5	.5	17	3.0	.2
8	4.0	1.0	18	3.0	.3
9	3.0	.8	19	4.0	.2
10	4.5	1.3	20	2.5	.1
Total	34.5	8.5	Total	32.0	2.0
Mean	3.45	.85	Mean	3.20	.20

and permit a mean flow of water through them of .85 cc. A like number of the infected stems have a mean diameter of 3.20 mm. and allow a mean water flow of .20 cc. If correction be made for the difference in diameter

of the infected and noninfected stems, then the water flow through the non-infected stems is reduced to .78 cc. On this basis, the reduction of water flow through the stems infected with *Phyt. stewarti* is 74.36 per cent.

DISCUSSION

The data here presented show that cucumber plants infected with *Erwinia tracheiphila* and those of sweet corn infected with *Phytophthora stewarti* behave similarly in their daily transpiration losses during the period of wilting. In all instances, the initial stages of wilting of the affected plants are accompanied by a decline in the transpiration rate, which progresses as the more advanced stages of wilting occur. In some of the wilting plants this reduction is evidenced earlier, or more markedly, than in others.

The reduction of the transpiration rate of the wilt-infected plants during the early stages of wilting is similar to what might occur were there an interference with the water absorption or conduction. The decline, in most instances, is marked and steadily decreases as wilting proceeds. Such a reduction would be in accord with the inability of the wilting plants to obtain enough water to maintain turgidity and would appear to be occasioned by a mechanical plugging of the xylem elements. None of the infected plants show an increase in their transpiration histories prior to or during the early symptoms of wilt such as that determined by Linford for the *Fusarium* wilt of peas. Rather, the decrease in the transpiration rate is in accord with the limited results of Ahmet, and of Yoshii and Masano.

Linford suggested that the increased transpiration recorded for wilting peas might result from the liberation of toxic filtrates by the fungus into the transpiration stream and, when transported to the foliage, might cause the leaf protoplasts to lose their water-retaining ability and induce wilt from an excess loss of water rather than from a deficiency.

The data show that the plants of cucumber and sweet corn have not wilted because of an excess loss of water. In no instance is there a marked increase of the transpiration rate immediate to or during wilting. Rather, the transpiration histories indicate that wilting is the direct reaction to insufficient water. The possibility, however, is not precluded that the toxic filtrates may decrease the permeability of the leaf protoplasts or else so affect their normal functioning as to induce wilting from lack of water.

The fluometric determinations made on wilting plants of both cucumber and sweet corn afford a further check for such a possibility. If the infected plants wilted as a result of leaf injury induced by toxic filtrates, expressed in either decrease or increase of the permeability of their protoplasts, then the stems of wilting plants should permit the passage of water through the xylem elements as readily as through those of the noninfected stems. Conversely, if wilting were occasioned by a mechanical plugging of the vascular system, then a proportionate reduction in the rate of water flow through the infected stems would be expected. The data fluometrically

recorded show that the water flow through those stems infected with *Erwinia tracheiphila* was reduced 82 per cent as compared with the noninfected stems. Those stems infected with *Phytomonas stewarti* showed a reduction of 74 per cent. Such a marked plugging would suffice to account for wilting from inability of the plants to obtain sufficient water.

However, the filtrates of both organisms, grown in beef-extract broth solution, readily wilted cuttings of both cucumber and sweet corn. In conformity with the toxic-filtrate theory for wilting, the fact that wilting was produced by the autoclaved as well as the nonautoclaved filtrates would further characterize the toxic principle as thermostable and of non-enzymatic nature.

A resumé of the literature shows that the results of investigations pertaining to the wilt phenomenon are not in accord, especially those based on the effects of toxic filtrates. Dastur (5) has criticized the postulate that wilting is attributed to toxic filtrates because plants or plant cuttings wilt when placed in filtrates. The investigations of Bisby (3), Barnum (2), Fikry (6), and of Dastur, also, show that the filtrates of some saprophytic fungi will induce wilting.

A common pathological and histological symptom of the two wilt diseases, caused by *Erwinia tracheiphila* and *Phytomonas stewarti*, is the occurrence of gum deposits in the xylem elements. In some instances, their presence would appear to be as important in plugging the transpiration stream as the bacterial organisms. Tyloses, which occur in some wilting plants, would contribute also to the plugging. If these gum depositions and tyloses are produced as a response of the host cells to the metabolic products of the pathogen, then, even such plugging action is, indirectly, induced by toxic filtrates.

From the results obtained in the present experiments, it is evident that the pathogens produce toxic filtrates, but the existence of the latter does not preclude the possibility of death resulting chiefly from a plugging of the vascular system. The results obtained from the transpiration histories of the wilting plants appear to indicate that wilting is in the main, induced by such a mechanical stoppage. These results are supported also by the fluometric determinations. Consequently, it is concluded that the primary cause of wilting of cucumber by *Erwinia tracheiphila* and of sweet corn by *Phytomonas stewarti* can be attributed chiefly to a mechanical plugging of the vascular tract rather than to the direct effects of toxic filtrates liberated by the pathogens.

SUMMARY

The wilt of cucumber infected with *Erwinia tracheiphila* and of sweet corn infected with *Phytomonas stewarti* has been investigated.

The data recorded for the transpiration histories of the plants infected with *E. tracheiphila* and *Phyt. stewarti* show a decrease in the transpiration losses during the initial stages of wilting.

The results fluometrically recorded for stems infected with these two organisms show a marked reduction for the water flow through these stems as compared with noninfected stems.

The filtrates of both *E. tracheiphila* and of *Phyt. stewarti*, from cultures of the organisms in beef-extract broth solution, induced wilting of cuttings of cucumber and sweet corn.

The transpiration and fluometric data obtained suggest that the wilting induced by *E. tracheiphila* and *Phyt. stewarti* is caused primarily by a mechanical plugging of the water-conduction system.

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THE TOXICITY OF CERTAIN CHEMICALS IN AQUEOUS SOLUTIONS TO SPORES OF *PENICILLIUM EXPANSUM*¹

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INTRODUCTION

Baker and Heald (2) recommended a 1-minute immersion of apples in a sodium-hypochlorite rinse, containing 0.4 per cent available chlorine, in order to control blue-mold decay. In commercial usage this recommendation has 4 disadvantages: The additional tank containing the sodium hypochlorite solution could be added to many apple washers only by remodeling the packing house; the odor of the chlorine gas given off from such a rinse is objectionable and workers find it irritating to the nasal passages and the eyes; the hypochlorous acid formed in solution corrodes the metal parts of the tank rapidly; and the presence of organic matter with which the chlorine reacts and the escape of chlorine gas from solution necessitate the frequent addition of sodium hypochlorite to keep the concentration constant. This sodium-hypochlorite rinse was the only chemical treatment known that would control blue-mold decay of apples, so an investigation was undertaken to find a chemical as efficient as sodium hypochlorite, without its drawbacks, from the commercial standpoint. In order to discover promising chemicals numerous laboratory tests were conducted. A summary of the experiments, using aqueous solutions of the chemicals, is presented in the present paper. The results must not be interpreted in terms of blue-mold-decay control but only as toxicity to spores, with indications for future work on decay control.

The chemical compounds, tested by various workers, for their fungicidal properties may be grouped as follow: (1) The phenates (14); (2) the dyes including the triphenylmethane dyes and others (7, 11, 12, 18, 19, 26, 27); (3) miscellaneous organic chemicals (5, 6, 8, 9, 10, 13, 16, 19, 22, 23, 25, 26, 28, 29, 30, 32, 33); and (4) inorganic compounds (1, 3, 4, 5, 15, 17, 19, 20, 21,

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22, 24, 27, 31). The literature dealing with the fungicidal properties of these compounds is voluminous and will not be reviewed in this paper. Briefly stated a survey of the published work has shown tests of 21 different dyes, 20 miscellaneous organic compounds, and 25 inorganic compounds, involving the use of various fungi.

The several workers have often obtained different results on the toxicity of the same chemical. A few of the variables that might account for these discrepancies are: temperature, length of exposure, concentration of the chemical, the presence or absence of organic matter, the hydrogen-ion concentration, and the organism tested.

In the experiments presented in this study an attempt was made to control all the factors mentioned above except hydrogen-ion concentration. This last factor was not examined in these tests, since, in commercial practice, known concentrations of chemicals must be added to washing solutions of definite composition; thus the hydrogen-ion concentration would not be a variable.

EXPERIMENTAL PROCEDURE

The spores of *Penicillium expansum* used in these experiments were from colonies grown 7 to 15 days on 2 per cent potato-dextrose agar in Petri dishes at 68° F., since Baker (1) has shown their resistance to chemicals to be greater than that of older spores. The spore suspension was obtained by placing an inch-square area of the culture of *P. expansum* in a flask containing 100 cc. of sterile distilled water. The flask was agitated vigorously for 4-5 minutes to free the spores from the agar and to break up the spore clusters as much as possible. Since the pieces of agar would have interfered with pipetting from this suspension, they were removed by means of sterile tweezers. A 1 cc. portion of this spore suspension was then pipetted into another 100 cc. water blank. The second spore suspension was allowed to stand for varying lengths of time, at the end of which a 1 cc. aliquot was transferred to a 9 cc. water blank. The 10 cc. of spore suspension thus formed was shaken, and, immediately afterwards, 1 cc. portions of it were placed in each of 3 tubes of cool (below 52° C.) liquefied agar, and the content of each tube was poured immediately into a Petri dish.

This method may seem indirect, but it was admirably adapted for the proposed experiments. The first spore suspension served as a stock solution, so that several experiments could be conducted consecutively without making a new suspension for each. The 100 cc. water blank, into which portions of the original spore suspension were transferred, contained the agent whose fungicidal action was being tested. A further dilution was accomplished by the transference of 1 cc. from the 9 cc. water blank to approximately 20 cc. of agar preparatory to plating. Tests of this procedure showed no reduction in viability of spores, no lessening of resistance to chemicals, and no residual chemical effect.

Preliminary experimentation showed that the use of spores from 1 sq. in. of the Petri dish culture in this procedure permitted the formation of

50 to 100 colonies on the resulting poured plates. This was sufficient to lend numerical weight to the results obtained and yet small enough so that individual colonies could be easily distinguished.

Early experimentation showed no increase in toxicity when the time of exposure was increased from $\frac{1}{2}$ to 2 minutes. This does not mean that length of exposure does not affect toxicity but that the difference between the effect of a 2-minute exposure and that of a $\frac{1}{2}$ -minute exposure was less than the difference between 2 consecutive concentrations, hence it could not be measured. For this reason 1-minute exposures were used for all experiments reported in the present paper.

THE PHENATES

The original interest in these chemicals came from the recommendations for the control of decay of apples made for one of them, sodium orthophenylphenate, by commercial concerns. Results of experiments conducted with two samples of sodium orthophenylphenate, one that had been prepared for 9 months and one that was newly prepared, and with two other phenates, sodium tetrachlorphenate and sodium-2-chlor-orthophenylphenate, are reported in table 2.

Table 1 shows an older sample of sodium orthophenylphenate to be much less toxic to spores of *Penicillium expansum* than a freshly-prepared sample. The 9-month-old sample was partially insoluble in water. This insoluble portion is probably a decomposition product formed on contact with the carbon-dioxide of the air. A mixture of sodium tetrachlorphenate

TABLE 1.—*Fungicidal effects on spores of Penicillium expansum exposed to various phenates at 68° F. for one minute*

Chemical used	Concentration in parts per million		
	1660	2500	5000
Sodium orthophenylphenate (old sample)	++++	++++	++++
Sodium orthophenylphenate (fresh sample)	+++	+++	-
Sodium tetrachlorphenate	+++	+++	+++
Sodium tetrachlorphenate + sodium 2-chlor-ortho- phenylphenate ^a	+++	+ ^b	+ ^b

- = no growth.

+ = 1-25% as many colonies as in check.

++ = 25-75% as many colonies as in check.

+++ = 75-125% as many colonies as in check.

++++ = over 125% as many colonies as in check.

^a A commercial mixture produced under a trade name—the relative amounts of each chemical are unknown.

^b Actually 4-6% as many colonies as in check.

and sodium 2-chlor-orthophenylphenate was superior to either sodium tetrachlorphenate or sodium orthophenylphenate alone. Unfortunately, sodium 2-chlor-orthophenylphenate was not available as an individual chemical; so no tests could be made to determine its toxicity.

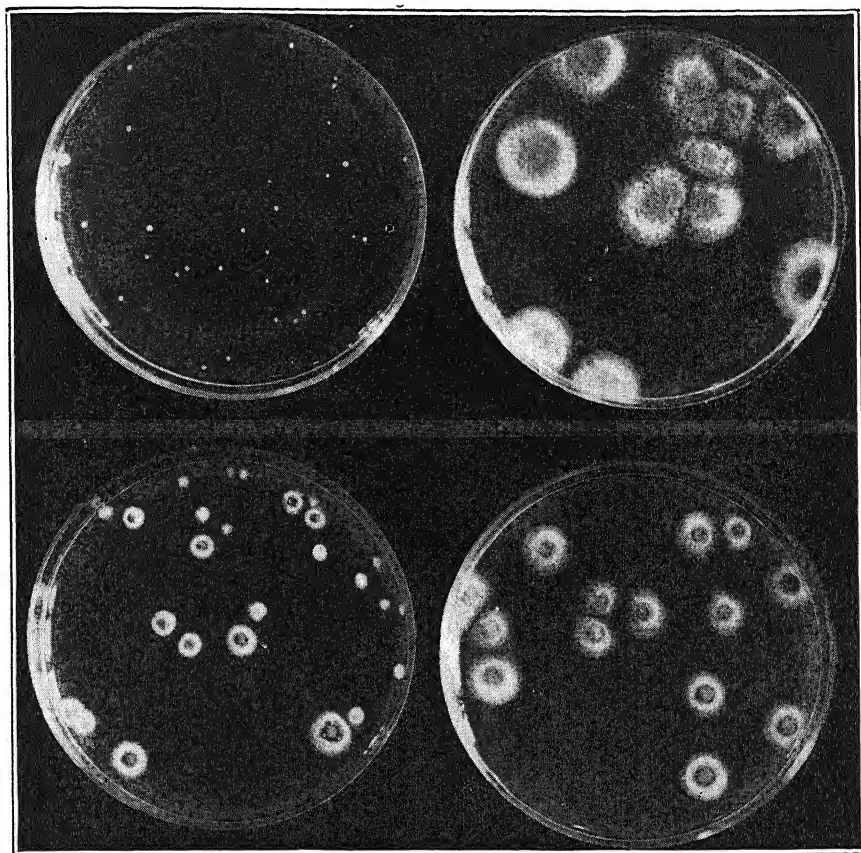


FIG. 1. Progressive inhibition of colony size in response to increases in concentration of sodium orthophenylphenate.

A progressive inhibition of colony size was noted, as the concentration of the sodium orthophenylphenate was increased, until complete prevention of colony formation was reached. A macroscopic examination of the obverse of these inhibited colonies showed numerous concentric rings. These rings were formed by the fungus through the alternation of spore and mycelial production. The center was raised and densely covered with spores giving a bull's-eye appearance to the colony when viewed from above. Figure 1 illustrates this quite clearly, as well as the progressive diminution in colony size. This was not merely a residual chemical effect, as shown by the normal development of colonies from spores seeded on agar that contained spores killed by sodium orthophenylphenate, together with the residual chemical itself. Neither was this phenomenon an induced genetical change, as spores from these inhibited colonies produced normal colonies.

The other phenates tested also caused an abnormal formation of the *Penicillium* colony on agar that became more marked as the concentration of the chemical was increased. However, these colonies were typified by

coremia and more vegetative growth than *P. expansum* normally produces on 2 per cent potato-dextrose agar. As in the case of sodium orthophenylphenate, experiments showed that this was neither an induced genetical change nor a residual chemical effect. Possibly, in both cases, the spore itself was injured sufficiently to produce an abnormal colony.

In commercial practice in the Pacific Northwest apples are not washed in cold solutions but at temperatures between 90° and 120° F. Thus it was decided to determine the effect of sodium orthophenylphenate at higher temperatures. For this purpose the flask containing the solution was brought to the required temperature in an oven before the spores were immersed. Though the immersion was made in the culture room, checks showed that the temperature of the solution dropped less than a degree during the course of the experiment. The results of this experiment are presented in table 3.

The data show that temperature has a marked effect on the efficiency of sodium orthophenylphenate, since a concentration of 3000 p.p.m. is toxic at 110° F. and 120° F., while a concentration of 4000 p.p.m. is required for toxicity at 68° F.

TABLE 2.—*Effect of temperature on toxicity of sodium orthophenylphenate solutions in one-minute exposures to spores of Penicillium expansum*

Concentration in parts per million	68° F.	110° F.	120° F.
200	+++	++	+++
400	+++	+++	+++
1000	+++	++	+++
2800	+++		
3000		—	—
3200	+++		
3600	+++		
4000	—		

— = no growth.

+ = 1–25% as many colonies as in check.

++ = 25–75% as many colonies as in check.

+++ = 75–125% as many colonies as in check.

TRIPHENYLMETHANE DYES

The fungicidal effect of these compounds has previously been tested largely on dermatophytic fungi. Their price (in C. P. grades at least) in any large concentration would prohibit their use in the apple industry. However, it was hoped that by testing some members of this series a relationship might be found between constitution and toxicity, and by this means other less expensive chemicals might be found.

Of the 9 dyes of this series examined, only crystal violet, gentian violet, and malachite green were toxic to spores of *Penicillium expansum*. All three of these compounds contain methyl groups attached to the amino-nitrogens. The least active—gentian violet—is an indefinite mixture of methyl derivatives. Malachite green has 4 methyl groups, and the most

TABLE 3.—*Toxicity of triphenylmethane dyes to spores of Penicillium expansum when exposed for one minute at 68° F.*

Chemical used (trade name)	Concentration in p.p.m.	
	100	1000
Acid fuchsin	++++	++++
Analine blue	+++	+++
Cotton blue	+++	++++
Crystal violet	++	—
Gentian violet	+++	+
Iodine green	+++	++++
Light green S. F.	+++	+++
Malachite green	++++	—
Methyl blue	+++	++++

— = no growth.

+= 1-25 per cent as many colonies as in check.

++ = 25-75 per cent as many colonies as in check.

+++ = 75-125 per cent as many colonies as in check.

++++ = over 125 per cent as many colonies as in check.

toxic—crystal violet—has 6 methyl groups. However, iodine green with 6 methyl groups was nontoxic to spores of *P. expansum* in the concentrations examined. It differs from the previous dyes in having a quaternary grouping on one nitrogen and this may explain the lack of toxicity. No theory can be developed from the limited data included here, but the results obtained support the theory advanced by Thornberry (26) that methylation increases the toxicity of dyes and phenylation decreases their toxicity.

An interesting investigation could be made to determine if the increase in colony number, such as results from exposure to acid fuchsin, is the result of a stimulation of germination or from a wetting effect that separates the spore clumps.

DYES OTHER THAN THE TRIPHENYLMETHANE SERIES

Table 4 gives the results obtained with miscellaneous dyes. Although Kobs and Robbins (11) have shown Eosin Y to be toxic to *Fusarium oxysporum* and Munro and Newton (19) found methylene blue to exert a fungicidal action on *Fusarium* sp., neither of these chemicals was effective in preventing the germination of spores of *Penicillium expansum* under the conditions of the present experiments. Table 4 also shows that none of the other miscellaneous dyes tested was toxic to spores of *P. expansum*.

MISCELLANEOUS ORGANIC CHEMICALS

Most of the chemicals tested in this group were selected because other workers had shown them to be fungicidal; some, such as Dichloramine-T, Halazone, and Metaphen, because of their common use as antiseptics; and a few were chosen at random. The results of experiments conducted with this group are reported in table 5.

Three of the compounds examined (salicylic acid, sodium salicylate and thymol) were sufficiently toxic to prevent germination of *Penicillium ex-*

TABLE 4.—*Action of dyes other than the triphenylmethane series when exposed to spores of Penicillium expansum for one minute at 68° F.*

Dye	Type	Concentration in p.p.m.	
		100	1000
Bismark brown	Azo	++++	+++
Eosin Y	Pyronine	+++	+++
Erythrosin	"	+++	++
Haematin	Flavone	+++	+++
Haematoxylin	"	+++	+++
Janus green	Azo	+++	+++
Martius yellow	Nitro	+++	+++
Methylene blue	Thiazine	+++	+++
Methyl orange	Azo	+++	+++
Neutral red	Azine-safranine	+++	+++
Nigrosine	" "	+++	++++
Orange G.	Azo	++++	++++

++=25-75 per cent as many colonies as in check.

+++ = 75-125 per cent as many colonies as in check.

++++ = over 125 per cent as many colonies as in check.

pansum spores under the conditions of the experiment. Various workers have tested the effect of these three compounds on *P. expansum* (5, 6, 11, 26, 32), since, according to Baker (1), the form commonly referred to as *P. glaucum* in reality belongs to *P. expansum*. However, all the investigators cited examined the action of these compounds while they were in continuous contact with the spores. The results obtained by the workers cited differed markedly in the minimum concentration found effective. This variation can be explained on the basis of the different media in which the chemicals were tested and on the pH of these media. The results presented in table 6 would indicate a relatively greater toxicity, since a concentration of 10,000 parts per million for either salicylic acid, sodium salicylate or thymol was effective in 1-minute exposures, whereas the experiments of the workers cited show concentrations varying from 200 p.p.m. to 43,000 p.p.m. necessary to prevent germination in continuous exposures. Further, Sabalitschka, and Dietrich (23) found a nutrient solution containing 2140 p.p.m. of salicylic acid or 43,000 p.p.m. of sodium salicylate to have no effect on germination of spores. These contradictory results emphasize the necessity of controlling and reporting all possible factors in toxicity experiments.

INORGANIC COMPOUNDS

The majority of the inorganic compounds used in the present investigation had been shown by other workers to possess fungicidal properties. The results of the experiments with these inorganic chemicals appear in table 6.

The two mercury compounds used, mercuric chloride and potassium mercuric iodide, were effective at a concentration of 1,000 p.p.m. However, unless the last trace of mercury could be removed after its application as a fungicide, these two salts could not be used on apples, since mercuric salts, even in minute quantities, are toxic to humans.

TABLE 5.—*The toxicity to Penicillium expansum spores of miscellaneous organic chemicals when exposed for one minute at 68° F.*

Chemical	Concentration in p.p.m.								
	10	40	80	100	160	200	400	1,000	10,000
Benzoic acid	++++ ^{a,c}							++	++
Chloral hydrate									++
Chloramine-T ^a									++
Dichloramine-T									+++
Gallie acid									+
Halazoned									+++
Hexylresorcinol				++		++			++
Mercurochrome									
Metaphen									
Oxalic acid								++	++
Phenol								++	++
Phloroglucinol									++
Picric acid									++
Pyrogallie acid									++
Resorcinol									++
Salicylic acid									++
Sodium salicylate									+
Succinic acid									++
Tartaric acid									++
Thymol								+	

- = no growth.

+ = 1-25 per cent as many colonies as in check.

++ = 25-75 per cent as many colonies as in check.

+++ = 75-125 per cent as many colonies as in check.

++++ = over 125 per cent as many colonies as in check.

^a Freshly prepared solutions were used to prevent loss of chlorine.^b Marked inhibition in colony size at this concentration.^c 10 p.p.m. saturated aqueous solution.^d Odd concentrations used since this came in form of pellet for water sterilization and 1, 2, and 4 pellets were used in 100 cc. of water.

TABLE 6.—*The fungicidal effect of various inorganic chemicals when exposed to spores of Penicillium expansum for one minute at 68° F.*

Chemical	Concentration in p.p.m.		
	100	1,000	10,000
Aluminum potassium sulphate		+++	+++
Barium nitrate		+++	++
Cadmium chloride		+++	++
Cerium oxalate		+++	+++
Chromium trioxide		++	—
Cupric acetate		+++	+++
Cupric ammonium sulphate	++++	+++	+++
Ferric nitrate		+++	++
Hydrogen peroxide		+++	++++
Iodine ^a		—	—
Lead acetate		+++	++++
Mercuric chloride		—	—
Nitric acid		+++	+++
Potassium chlorate	+++	++++	+++
Potassium dichromate		+++	—
Potassium iodide	+++	+++	+++
Potassium acid sulphate	+++	+++	++
Potassium mercuric iodide		—	—
Potassium permanganate	+++	+++	++
Potassium sulfite	+++	+++	+++
Sodium carbonate		+++	++
Sodium chloride	+++	+++	+++
Sodium acid sulfite	++++	+++	++
Sodium thiosulphate	+++	+++	—
Sodium tetraborate	+++	+++	+++

—=no colonies.

+ = 1-25 per cent as many colonies as in check.

++ = 25-75 per cent as many colonies as in check.

+++ = 75-125 per cent as many colonies as in check.

++++ = over 125 per cent as many colonies as in check.

^a Dissolved by means of KI.

Iodine also prevented germination of *Penicillium expansum* spores at a concentration of 1,000 p.p.m. Nattrass (20) has shown that iodized wraps reduced citrus decay caused by *P. italicum* and *P. digitatum*. Since the tests reported in table 6 show that it is also exceedingly toxic to *P. expansum* further investigation of its fungicidal properties when incorporated in apple wraps should be undertaken. Iodine is too expensive to be used profitably as a fungicidal rinse for control of blue-mold decay of apples.

Chromium trioxide, potassium dichromate, and sodium thiosulphate were less toxic than the 3 chemicals previously described but were fungicidal at concentrations of 10,000 p.p.m.

Chromium compounds have been little used as fungicides, but in recent years several workers (3, 17, 19, 21, 24 and 27) have shown their toxicity to fungi. Preliminary work with sodium chromate, sodium dichromate, ammonium dichromate, potassium chromate, chromium trioxide, and potassium dichromate indicates that the toxicity of these compounds is proportional to the normality of the solution with respect to chromium and has no relation to the chromate or dichromate state.

SUMMARY

In the absence of organic matter a concentration of 4000 p.p.m. of pure sodium orthophenylphenate kills spores of *Penicillium expansum* in one minute at 68° F., while at 110° F. under the same conditions a concentration of 3000 p.p.m. of this chemical is effective. Since this compound decomposes with exposure to air, the solubility of each lot should be tested before deciding on its value.

A mixture of sodium tetrachlorophenate and sodium 2-chloro-orthophenylphenate¹ exhibits greater toxicity to spores of *P. expansum* in one-minute exposures at 68° F. than does either pure sodium tetrachlorophenate or sodium orthophenylphenate. Under the experimental conditions a concentration of 2500 p.p.m. of such a mixture was sufficient to kill a high percentage of *P. expansum* spores.

Of nine triphenylmethane dyes examined, only crystal violet, gentian violet, and malachite green were toxic to spores of *P. expansum*. The limited evidence of the present experiments indicates a relationship between the number of methyl groups attached to the amino-nitrogens and the fungicidal activity.

None of the 12 miscellaneous dyes tested showed any fungicidal activity in the highest concentrations used (1000 p.p.m.).

Sodium salicylate and thymol were the only two, of 20 miscellaneous organic fungicides examined, that completely inhibited colony formation in concentrations of 10,000 p.p.m.

Twenty-five inorganic chemicals were tested. Chromium trioxide, potassium dichromate and sodium thiosulphate were fungicidal at a concentration of 10,000 p.p.m. Iodine, mercuric chloride, and potassium mercuric iodide, were effective in concentrations of 1000 p.p.m.

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¹ Sold under a trade name.

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A NEEDLE-CAST OF DOUGLAS FIR ASSOCIATED WITH *ADELOPUS GÄUMANNI*¹

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Douglas fir, *Pseudotsuga taxifolia* (Lam.) Br., is, economically, the most important single tree species in North America. Because of its rapidity of growth it has been widely planted in western Europe, where there has been a constant attempt for over 2 centuries to introduce additions to the relatively few and slow-growing native species with their wood of restricted technical value. Exotics are always exposed to new diseases that may prove disastrous not only to the tree in its new environment, but may also be introduced into its native range with unfortunate consequences. This has been the history of eastern white pine (*Pinus strobus* L.) and the blister-rust fungus (*Cronartium ribicola* Fisch.). Consequently, when a new disease of an important American tree is reported abroad it is imperative that it be given serious consideration.

HISTORY AND DISTRIBUTION

In 1925 a new needle-cast of Douglas fir was found on approximately 20-year-old trees of the green form of the species at Hardern near Lyss, Canton of Bern, Switzerland (6). It was suggested that the causal fungus was possibly *Adelopus balsamicola* (Peck) Theissen, a common saprophyte on dead needles of various species of *Abies*, widespread in the United States, where it was first described in 1881, and in Europe, where it was first reported in 1907 (17, p. 170). The fungus next appeared on Douglas fir in southern Germany, near Lake Constance, in 1931 (5, p. 37), and, in what was Austria, in 1934 (16), apparently carried from Switzerland by the southwest winds, prevalent during spring and summer. *Rhabdocline pseudotsugae* Sydow, which is prevalent on Douglas fir in southern Germany, has not yet appeared in Switzerland, apparently because it must spread in that direction against the winds prevailing during the period of spore dispersal.

The fungus was named *Adelopus balsamicola* forma *Douglasii* by Steiner (17) and *A. güumanni* by Rohde (13), who considered it sufficiently different from the saprophytic *A. balsamicola* on *Abies* to warrant its description as a new species. Petrak (12) transferred the fungus to the genus *Phaeo-cryptopus* on the grounds of priority and agreed that the fungus warranted specific rank, thus naming it *P. gaeumanni* (Rohde) Petrak. The fungus is now distributed throughout Switzerland (18) and has spread north into southern Germany as far as Freiburg-im-Breisgau, Stuttgart and Passau (14). The fungus has not been recorded in northern Germany,

¹ The observations in Europe were made during the period from February through August, 1939, while the writer was a fellow of the Oberlaender Trust of the Carl Schurz Memorial Foundation. Grateful acknowledgment is made to that organization.

where, in the summer of 1939, the writer examined a number of Douglas fir stands without finding the organism. However, the fungus has recently been found in 3 localities in Denmark (3), so it will probably appear in northern Germany sooner or later. It is not known in The Netherlands (19), although Douglas fir has been extensively planted there.

The fungus is prevalent in the British Isles. It was first recorded by Wilson and Waldie (20, p. 152) under the name of *Adelopus balsamicola* on Douglas fir in Devon, England, in 1927 and in Antrim, Ireland, in 1928, but no mention of associated disease was made. Since then, the fungus has been found at several places in Great Britain, although the effect on the host usually is not severe (1); but, in Ireland, the fungus is both prevalent and quite severe (10).

The organism was first found in the United States in January, 1938, at East Willington, Connecticut, by de Caprio,² although it was actually in Connecticut prior to 1929 (11). The affected Douglas firs at East Willington comprised a small plantation about 20 years old. In September, 1938, the fungus was discovered in another plantation near Dover, New Hampshire, by Hansbrough.³ By February, 1940, it was known from 6 localities in Connecticut, 1 in Rhode Island, 2 in Massachusetts, 2 in Vermont, 3 in New Hampshire, and 2 in Maine.

Within the natural range of Douglas fir in western North America the fungus has been present for many years, although it passed unnoticed until Wilson⁴ visited that region from April to August, 1938 (9, p. 218), because there the fungus is either not at all or so negligibly injurious to the host that it is easily overlooked. Since then the fungus has been found at such widely separated localities in British Columbia, Washington, and Oregon⁵ that it must be considered generally distributed, although harmless in the Douglas fir region of the Pacific Coast. It also has been reported from New Mexico.⁶ An examination of old collections of Douglas fir needles in the herbarium of J. S. Boyce showed that infected specimens had been collected in Josephine County, Oregon, in 1916 (No. 359), in Lane County, Oregon, in 1921 (No. 968), and in Shasta County, California, in 1923 (No. 392), but the presence of *Adelopus gümanni* was not observed at the time. The collections were made because *Rhabdocline pseudotsugae* was on the needles. Both *A. gümanni* and *R. pseudotsugae* may develop on the same needle.

Specimens from Connecticut and specimens and cultures from Oregon were studied by Rohde and specimens from Connecticut and Oregon were studied by Wollenweber.⁷ In addition, the latter made observations in

² A. de Caprio, Department of Entomology, Connecticut Agricultural Experiment Station, New Haven, Conn.

³ J. R. Hansbrough, Division of Forest Pathology, Bureau of Plant Industry, New Haven, Conn.

⁴ Malcolm Wilson, Royal Botanic Garden, Edinburgh, Scotland.

⁵ Meinecke, E. P. The *Adelopus* needle-cast of Douglas fir on the Pacific Coast. 5 pp. (mimeo.) Sacramento, Calif.: Dept. Nat. Resources, Div. Forestry. 1939.

⁶ Gill, L. S. File Memorandum. Observations on *Adelopus* in the Lincoln National Forest in 1939. 3 pp. (typewritten ms.). Oct. 11, 1939.

⁷ H. W. Wollenweber, Biologische Reichsanstalt, Berlin-Dahlem, Germany.

the Douglas fir region of the Pacific Northwest during late summer of 1939. Both authorities have pronounced the fungus to be *Adelopus gümmani*.

In Europe the disease has been called *Adelopus* or Swiss needle-cast of Douglas fir (*Adelopus* or Schweizer Douglasiennadelschütte) to distinguish it from *Rhabdocline* needle-cast caused by *Rhabdocline pseudotsugae*, which is common in northern Europe but not yet recorded from Switzerland.

HOST

The only host so far known is Douglas fir.

Although in the United States it is recognized that there are differences between *Pseudotsuga taxifolia* (Lam.) Br. (*P. douglasii* Carr.) on the Pacific Coast, in the Intermountain Region, and in the Rocky Mountain Region, these differences are considered to indicate growth forms or climatic varieties (4). Europeans, however, often consider Douglas fir in America to comprise 2 species and 1 variety, that is, *P. taxifolia* for the coast form, *P. taxifolia* var. *caesia* Schwer. for the intermountain form, and *P. glauca* Mayr for the Rocky Mountain form (7). Others recognize but one species, *P. taxifolia*, considering, however, that it comprises three botanical varieties, viz, *viridis*, *caesia*, and *glauca*. Various common names have been used for these growth forms, species or varieties, such as coast or green Douglas fir, intermountain, transition, Fraser River or gray Douglas fir, and Rocky Mountain or blue Douglas fir. The most suitable names are coast, intermountain, and mountain Douglas fir. The names based on the color of the foliage are not appropriate since individual trees of the intermountain and mountain forms may have foliage just as green as coast trees; while Fraser River is a decided misnomer, since all 3 forms occur along its extensive length.

Although differences between the coast and the mountain form are in general well marked, it is often difficult to distinguish the intermountain form from the coast form, on the one hand, or the mountain form, on the other. The most reliable difference depends on a microscopical examination of the leaf, in that idioblasts are lacking in the coast form, are few in the intermountain form, and abundant in the mountain form (7, p. 87). Idioblasts are large, stellate cells of unknown function among the ordinary parenchyma cells of the leaf.

Douglas fir in Europe has previously shown decided racial differences in its reaction to parasites. *Adelges cooleyi* Gill. has so far been confined to the coast form of the tree, while *Rhabdocline pseudotsugae* has been limited to the intermountain and mountain forms, although, in the United States, both the insect and the fungus occur on all 3 forms. *Adelopus gümmani*, as seen in Switzerland and southern Germany, is not so selective. The intermountain and mountain forms are severely attacked, but the coast form, although infected, on the whole has not thus far been so severely injured. It is yet impossible to state whether the coast form is actually more resistant in the end; but, certainly, where it was possible to compare

immediately adjacent trees or stands of the various forms, the fungus had developed much more slowly on the coast form. Unfortunately, it is not always easy to separate the forms without careful study; the actual origin of the seed for most Douglas fir stands in Continental Europe is unknown, much of it having been purchased from commercial seedsmen, so that rather severely infected stands, thought to be of the coast form, may actually be the intermountain variety. If the coast form is resistant and the mountain form highly susceptible, it would be expected that the intermountain form should in some instances show a certain resistance, in others considerable susceptibility. The fungus was first found in 1939 in the plantations at Kaiserslautern in the Bavarian Pfalz established from seed of known provenance, collected in various localities throughout the United States and Canada, so that within a few years a definite comparison between the reactions of the 3 forms should be possible.

It also was apparent, in Europe, that either there was a marked variation in resistance of individual trees or that a number of plantations had been established from seed of mixed origin, *i.e.*, of 2 or 3 of the growth forms, because of immediately adjacent trees one might be heavily infected and its neighbor lightly or not at all. Probably both conditions exist. Individual variation in their reaction to other pathogenic insects and fungi on the needles is such a usual phenomenon among conifers that it also most likely occurs with Douglas fir in relation to *Adelopus gäumannii*. In seeking resistant types for future propagation, individuals will be just as or possibly more important than forms or varieties as a group.

In western United States and Canada the fungus has been collected commonly on the coast form, occasionally on the intermountain form, and rarely on the mountain form. However, this relative prevalence is based merely on the amount of collecting that has been done and is no criterion of the distribution of the parasite, since no systematic search for it has been made throughout the range of the host.

In the eastern United States the infected plantations have been of the intermountain and mountain forms, as judged from ocular observation. Most of the plantations were too young to bear cones, and the needles have not yet been examined for idioblasts. Actually, the coast form must be quite rare in the East, since the climate is too severe for it.

DAMAGE

In Europe the fungus causes casting or shedding of the needles in varying degrees beginning with the oldest (Fig. 1), but when an attack is really severe, by the middle of the growing season only the needles of that season remain on the twigs together with some needles of the previous season. Ordinarily, in Europe, the needles of Douglas fir are retained for about 5 years or more. When this heavy loss of foliage continues for several years the trees become moribund and finally die, death often being hastened by *Armillaria mellea* (Vahl.) Quel., invading the root collar. Gen-

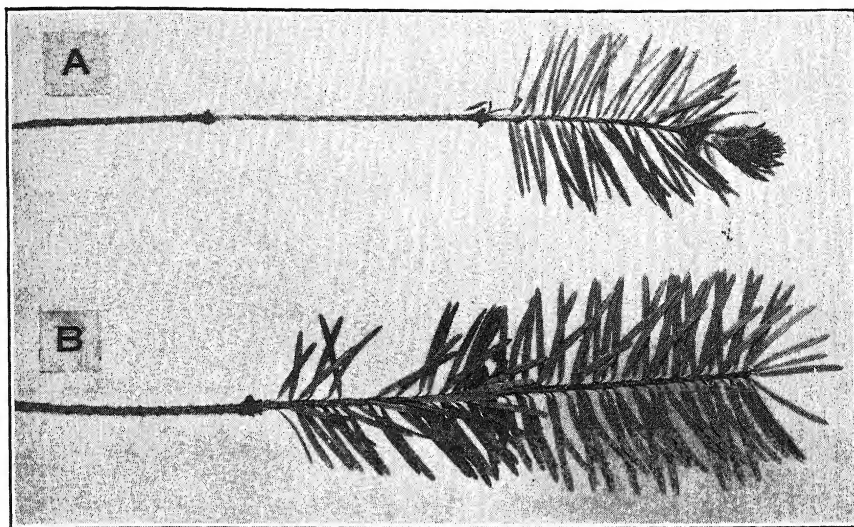


FIG. 1. Effect of *Adelopus gäumanni* on Douglas fir. A. A twig collected at Rapperswil, Switzerland, May 23, 1939. Only 1-year-old needles remain, all older ones having been killed and cast. B. A twig collected at East Willington, Connecticut, May 3, 1938. 1-year-old and some of the 2-year-old needles remain.

erally the infected trees are not allowed to die naturally but are cut and utilized. A number of plantations, although small in area, have already been ruined.

Young plantations have suffered most, but, as the infection becomes longer established in a locality, progressively older stands are involved. The oldest infected stand seen was of the coast form, 55 years old, but it had not yet been injured, since it was growing vigorously in both height and diameter. The most alarming picture was presented by a 47-year-old stand in the Forest of Emmendingen near Freiburg. This stand had the general characteristics of the coast form, including cones without reflexed bracts, but it may have been the intermountain form. The trees, varying from 14 to 18 in. in diameter at breast height and from 85 to 90 ft. tall, were of excellent form with little taper and a long, clear length. The foliage, however, was generally infected and many needles had been cast, so that the crowns were rather thin when viewed from below, thus permitting plenty of light to reach the forest floor, which, under normal conditions, would have been completely shaded. No trees had died yet in the stand, but cross sections of several stems showed that diameter growth had been sharply reduced in 1934; since then the growth rings were barely perceptible.

The situation is such that, in southern Germany, planting of Douglas fir has stopped, while in Switzerland there is a little more optimism; planting still continues but only in mixed stands. Actually the loss of Douglas fir in southern Germany and Switzerland is not so serious because native conifers make a satisfactory rate of growth. But the tree is highly important to The Netherlands and northern Germany, where European con-

fers grow slowly and often are not vigorous. The drier climate of these latter regions, however, may prevent the disease from being consequential, although it seems certain that the fungus will ultimately appear there. In Ireland the damage apparently approaches that on the Continent (10), but in Great Britain reports do not indicate such severe injury (1, p. 70).

In the eastern United States the damage is about equal to that in some of the less severely infected but still damaged stands in southern Germany and Switzerland. In the Douglas fir region of the Pacific Coast, even though the fungus is prevalent, it has caused no injury.⁸ Some injured stands, both in Europe and the eastern United States, have also been invaded by *Rhabdocline pseudotsugae* and *Adelges cooleyi*, so that it is hard to estimate the degree of injury by *Adelopus gäumannii* in all instances.

SYMPTOMS

The most conspicuous symptom of severely attacked trees is the exceedingly thin foliage resulting from the loss of so many needles. In some cases only 1-year-old needles and those of the current season remain. The foliage also has a general yellowish to brownish appearance. Epicormic shoots may develop in response to the greater amount of light available within the crown.

On closer examination the 1-year-old and older needles will be seen to vary from yellow-green or mottled yellow-green to mottled brown or completely brown, depending on the degree and length of time of infection. Complete browning usually occurs just before the needles drop. Examination of the under surface of diseased needles with a hand magnifier will show the numerous black perithecia of the fungus appearing as two soot-like black streaks, one on each side of the middle nerve (Fig. 2). This is the most reliable symptom of the disease.

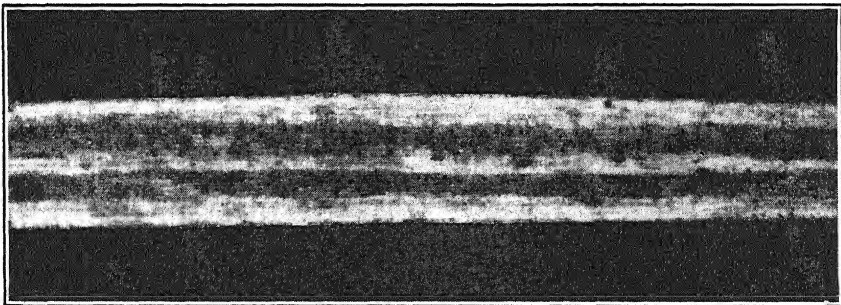


Fig. 2. Perithecia of *Adelopus gäumannii* on the under surface of a Douglas fir needle. About $\times 15$.

THE CAUSAL ORGANISM

The fungus *Adelopus gäumannii* Rohde or *Phaeocryptopus gaeumannii* (Rohde) Petrak (Ascomycetes, Capnodiaceae) is considered to be the

⁸ See footnote 5.

casual agency because of its constant association with the disease. The pathogenicity of the fungus has not yet been proved by inoculations.

The development of the fungus in southern Germany and Switzerland has been investigated by Rohde (13, 14). Ascospores mature and are disseminated in May and June, infecting the nascent needles. Indications are that older needles also can be infected, although possibly only to a limited extent. Experiments are now under way in Europe to determine this. Intercellular mycelium develops in the palisade and mesophyll tissues of the leaf during summer and fall (17, p. 178), but there is no outward evidence of its presence until winter when the waxy plugs in the stomata are pushed away by the rudimentary perithecia, giving the under surface of the needles a greener color. Later in the winter and in spring, isolated perithecia issue from the stomata (Fig. 2). However, in 1938, in Connecticut, the first indications of perithecia on needles of that season were observed on November 4. In southern Germany asci are formed in early May, while the spores begin escaping about 2 weeks later. During the second summer infected needles become yellow-green or mottled yellow-green; some may become brownish. During the second winter most of the infected needles become somewhat brown, or mottled brown, and more perithecia appear. During the third summer and the following winter the brown color intensifies, more new perithecia appear, and, after spore discharge from these in May and June, the needles turn completely brown and drop. Thus a single needle may bear perithecia, formed during 3 successive seasons. The behavior of the infected needles, however, is not uniform because some die and drop following maturity of the second crop of perithecia when the needles are about 2 years old, while on certain trees all 2-year-old needles may be cast, leaving only 1-year-olds and those of the current season. Again, fully developed perithecia may be found on apparently normal green needles showing no discoloration. Whether the perithecia that remain on the needles for 2 or 3 successive seasons produce asci and spores, except during their first season, is not known.

Mature perithecia are black and spherical, from 0.04 to 0.1 mm. in diameter, with a flattened base resting close on the needle to which they are attached by a short, conical, black stipe occupying the outer funnel-shape space between the guard cells of the stomata. There is only 1 perithecium per stoma. In a perithecium there are about 15 clavate asci, each with 8 hyaline bicellular, somewhat ellipsoid spores, constricted at the septum. The upper cell of the spore is somewhat broader than the lower. Paraphyses are lacking. No other fruiting stage of the fungus is known.

ORIGIN OF THE FUNGUS

The origin of the fungus is, for the present, and perhaps must remain for all time, in the realm of pure speculation. There are, however, several theories that demand evaluation.

1. The fungus in Europe and the northeastern United States is a new pathogenic strain that has arisen from some weak parasite or from a

saprophyte, such as *Adelopus balsamicola*, by hybridization between existing strains or by mutation.

This would presuppose that the new strain had arisen more or less simultaneously in the British Isles, in central Europe, and in the northeastern United States, or that it had appeared in only one region and had then been introduced to the others quickly.

2. *Adelopus gäumannii* in Europe, although morphologically identical with the fungus in the Douglas fir region of the Pacific Coast, differs from it in being pathogenic and is a strain introduced into Europe from some other part of the world, possibly Asia.

Granting this, however, one must also conclude that *Adelopus gäumannii* in the northeastern United States, where it is also pathogenic, also differs from that on the Pacific Coast and has been introduced either from Europe, Asia, or elsewhere. Since the fungus is known to have been in New England about the same length of time as in Europe it seems doubtful that it came from there.

3. *Adelopus gäumannii* is not a valid species but merely *A. balsamicola* attacking Douglas fir. *A. balsamicola*, heretofore known only as a saprophyte, or possibly a weak pathogen on *Abies*, has become seriously pathogenic on Douglas fir in Europe and to a lesser degree in the northeastern United States, where the tree is an exotic and, consequently, more susceptible to disease.

However, it is difficult to believe that *Adelopus balsamicola*, for decades a harmless saprophyte on *Abies* in North America and Europe, should suddenly become pathogenic on Douglas fir in Europe and the northeastern United States. Furthermore, since this fungus presumably has been known for so many years in Europe, it seems unlikely that it would have remained a saprophyte for so long on *Abies* before rather suddenly becoming pathogenic on Douglas fir, particularly since this tree has been planted in Europe for over a century. In addition, it cannot be accepted that *Adelopus balsamicola*, as known in Europe since 1907, was introduced from North America before carefully checking up the hosts and range of this species, the involved synonymy of which has recently been given by Petrak (12) under the name of *Phaeocryptopus nudus* (Peck) Petrak. For example, *P. abietis*, one of the synonyms, was described from Russia by Naoumov. It seems to me that *Adelopus balsamicola*, or *Phaeocryptopus nudus* as Petrak states it should be called, is probably native to both North America and Europe as a saprophyte, so that it has existed in Europe along with Douglas fir since this tree was introduced.

Finally, mycologists are now agreed that *Adelopus balsamicola* on *Abies* and *A. gäumannii* on *Pseudotsuga taxifolia* are separate species. The differences between them seem too great to be explained by the reaction of one species of fungus to two species of hosts.

4. *Adelopus gäumannii*, native to the western United States and Canada, where it occurs on Douglas fir as a practically harmless parasite, has

been introduced into the northeastern United States and Europe where it has become pathogenic and injurious, because Douglas fir in these regions is an exotic with consequent increased liability to disease and because climatic conditions in these regions are more favorable to the development of the fungus.

On the Pacific Coast, late-spring and early-summer precipitation, when the fungus is discharging its spores, is usually meager or almost lacking, whereas during the remainder of the summer and early autumn there is generally only an occasional shower or a complete lack of rainfall. Temperatures are rather high. In the northeastern United States, spring and summer rains are of frequent occurrence, usually in the form of heavy to torrential showers or, occasionally, extended periods of rainfall lasting from one to several days. Meanwhile, there is also considerable bright, sunshiny weather with relatively high temperature. In southern Germany and Switzerland, during the same seasons, showers are an almost daily occurrence, the sky is frequently cloudy and the temperature nearly always moderate. Ireland has a similar climate.

A change in climate may affect profoundly the development of a parasitic organism. *Rhabdocline pseudotsugae* has developed more prolifically in Europe than in its drier and warmer native home in western North America. *Adelges nüsslini* Börner is destroying all the *Abies nordmanniana* Spach., in southern Germany and the tree can no longer be grown there. Both the tree and the insect were introduced from the Caucasus, where *A. nordmanniana* is said to develop satisfactorily in spite of the insect on it. The explanation is said to be that the Caucasian climate is too wet for the insect to develop vigorously; consequently, the tree can tolerate it, but in the generally drier climate of southern Germany the insect develops so vigorously that the tree is overcome. Certainly long wet periods between May and October, which occur occasionally in southern Germany, cause high mortality among the insects, while those localities with an annual precipitation of more than 1100 mm. are usually not infested (8), although this last finding is disputed (15). Hence it seems not unreasonable to assume that *Adelopus gümmanni* has changed from a harmless parasite on the Pacific Coast to a pathogen in the different climates of the northeastern United States and Europe.

The wide distribution of the fungus on the Pacific Coast indicates that *Adelopus gümmanni* is indigenous on Douglas fir in that region. There is no indication, as is usually so with introduced parasites, that it has spread from one or more centers. Its wide distribution, but in scattered plantations in New England, could be accounted for by a direct introduction on nursery stock from the West to one or more eastern nurseries and its redistribution to various plantings. Both *Adelges cooleyi* and *Rhabdocline pseudotsugae* on Douglas fir have reached the East in some unknown way, presumably on living plant material brought from the West.

In central Europe the fungus started from one definite locality in western Switzerland. Since Switzerland has no regulations affecting the

importation of living plant material, and, since the country is a mecca for visitors and temporary residents from all over the world, many possibilities for introduction exist. The disease in Great Britain and Ireland may be the result of one or more introductions of the fungus from North America, while the central European infection may have originated by introduction to Switzerland directly from western North America or *via* Great Britain. *Adelges cooleyi*, *Rhabdochline pseudotsugae* and *Keithia thujina* Durand have all reached continental Europe from western North America, although the exact manner by which they bridged the gap is not known. Two of these at least probably came by way of Great Britain, but how they were introduced there is not definitely understood. The three infections in Denmark are thought to have originated from spores carried by westerly winds from the British Isles (3). In view of past developments the introduction of *Adelopus gäumannii* from North America into Europe may be regarded as a logical and not as an unexpected phenomenon difficult to believe.

CONTROL

No method of control has been developed. German foresters are considering the possibility of resistant races or individuals. The Swiss are planting Douglas fir in mixture only now, not with the idea that mixed stands as such will check the disease but rather on the basis that if the disease proves over the years to be as catastrophical as it now appears other species will form the stand when the Douglas fir is eliminated.

SUMMARY

A new needle-cast disease of Douglas fir (*Pseudotsuga taxifolia*) known as Swiss needle-cast or *Adelopus* needle-cast appeared in Switzerland in 1925. Since then the disease has been found in southern Germany, the former Austria, the British Isles, and Denmark. The disease also occurs in the New England States. Although the causal organism is present on living Douglas fir on the Pacific Coast the trees are not diseased by it. Douglas fir is the only host, but the incidence of the disease is variable enough to indicate a possibility of resistant growth forms or individuals. Severe infection results in progressively increasing defoliation followed by death of the tree. *Adelopus* (*Phaeocryptopus*) *gäumannii* is constantly associated with the disease. Four theories as to the origin of the fungus are evaluated. No method of control is yet known.

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THE EFFECT OF CERCOSPORA BETICOLA ON THE CHEMICAL COMPOSITION AND CARBON ASSIMILATION OF BETA VULGARIS¹

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Cercospora beticola Sacc., causing leaf spot of sugar beet, is the most important and destructive fungus attacking that crop plant in the United States. The pathogen not only causes losses in tonnage and sucrose content but also affects the purity of the juice derived from diseased beets.

With the exception of sucrose, little, however, is known regarding the chemical changes or the yields of certain products induced within the host

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cells and tissues by this fungus. It is the purpose of this paper to present data showing the comparative effect that the *Cercospora* leaf-spot organism has on certain carbohydrate and nitrogenous compounds within the roots and foliage of diseased and healthy sugar-beet plants.

METHODS

The samples for these experiments were collected under greenhouse conditions and from the field at Kanawha, Iowa. In the greenhouse series 100-g. samples were collected, while in the field series 200-g. samples were used. In the greenhouse tests the roots were sliced transversely in thin slices, weighed, and then dropped into 450 cc. of boiling 95 per cent alcohol; in the field tests a wedge-shape sample was removed from the roots of beets with a Diston beet rasp. The sample of pulp was obtained by first cutting the root longitudinally in half and then passing it through a Diston rasp and removing a V-shape portion in the form of pulp, which was used in making the composite samples. The pulp from the 10 beets, comprising a composite sample was thoroughly mixed, weighed, and dropped into 800 cc. of boiling 95 per cent alcohol. Six replicate samples of roots and 3 of crowns, using healthy, pruned, and diseased sugar beets, were used in the greenhouse series, while 3 samples of a composite of 10 beets each were used in the field experiments. In all cases the samples were prepared from the plants as soon as they were pulled from the soil.

The methods presented in the text by Loomis and Shull (3) were followed in the analytical work. The material was extracted by decantation, 14 extractions being made. When the extractions were completed the 100-g. samples were made to a volume of one liter, and the 200-g. samples up to 2 liters. The Munsen-Walker-Bertrand method was used for reducing and total sugars, while fructose was determined by Jackson and Mathews' modification of Nyn's selective method. Sucrose was inverted by invertase. Pectin was determined as crude calcium pectate.

Insoluble nitrogen was determined on the extracted residue, after pulverization in the ball mill, by the unmodified Kjeldahl method. Total soluble nitrogen including nitrates was determined on the alcoholic extract by the reduced-iron method.

Soluble solids were determined on an aliquot of alcoholic extract dried at 90° C. for 48 hours. The insoluble solids represented the dry weight of the material not extracted with 80 per cent alcohol.

The gas-stream method as modified by Heinicke and Hoffman (1) was used to measure CO₂ absorption by leaves. The air was removed at a rate of 85 l. per hour from a point about 6 cm. beyond the apex of leaves enclosed in water-proof cellophane bags, open at the base. The quantity of CO₂ removed from the air per 100 sq. cm. of leaf surface was determined by comparing with controls run on the air. The control run on the air consisted of placing a cellophane bag over the tube used to withdraw the gas from the bags for analysis. Leaf area was determined by tracing around

the leaves and measuring the area with a planimeter. In general, the leaves did not remove over 15-20 per cent of the CO₂ from the air. In taking measurements on CO₂ absorption by leaves, both healthy and diseased specimens were used. The amount of necrosis present on the various classes of diseased leaves was as follows: healthy, no visible signs of leaf-spot symptoms; class 2, 10-50 spots; class 3, 50-100; class 4, 100 to slight coalescence of symptoms; class 5, 50 per cent of leaf area necrotic; class 6, complete necrosis of lamina.

The healthy and pruned plants grown in the field experiment were sprayed frequently with Bordeaux mixture 4-4-50 to prevent infection by *Cercospora beticola*. The average number of leaves produced by these plants was 32. An average of 22 leaves per plant was defoliated by the pathogen in the nonsprayed series. A like number of leaves was removed from plants in the treated series by pruning near the crown. The pruning was performed intermittently on the same plants so as to correspond with the rate of defoliation induced by the pathogen on the nontreated series. The average number of leaves produced by the plants in the greenhouse series was 28, and an average of 18 leaves was defoliated following inoculation with *C. beticola*.

EXPERIMENTAL RESULTS

Roots and Crowns

Total solids or total dry matter was divided into alcohol-soluble and alcohol-insoluble fractions. The total solids in healthy roots and crowns were higher than in similar tissues from diseased plants (Tables 1 and 2). In table 1, with greenhouse material, the pruned roots and crowns were lower in percentage total solids than were the healthy, while in table 2 there was little difference between the pruned and the healthy. In addition to the healthy plants, used as controls, a second set of healthy plants was included that had the leaves removed or pruned, similar in number of leaves to those destroyed by the *Cercospora* leaf-spot organism on the diseased plants. The effect of pruning was, however, never so severe as the disease in reducing total solids. The relation of the insoluble residue followed the same general pattern as did the total solids, except the pruned plants were always intermediate between the healthy and the diseased. The crowns invariably contained a greater amount of insoluble material than did the roots. The trend of the soluble solids was similar to that of the total solids.

The soluble solids were divided into several fractions. The main constituent in sugar-beet roots and crowns is sucrose, while small amounts of glucose and nitrogenous compounds are present. In the greenhouse plants healthy roots had a sucrose percentage of 15.27, while, in diseased roots, it was 12.08, indicating that the leaf-spot organism caused a reduction of more than 20 per cent. Sucrose was reduced from 13.63 per cent in healthy crowns to 11.18 per cent in diseased crowns, a reduction of more than 17 per cent. In the field-grown plants the percentage reduction was less.

Pruning had some effect on the percentage of sucrose, but not so much as did the disease.

Analyses of the reducing sugars showed that fructose was absent from sugar-beet roots and either absent or present in very slight quantities in the crowns. The reducing substances present within the roots are considered to be glucose. There appears to be no consistent relationship between the healthy, pruned, and diseased plants with respect to glucose content.

TABLE 1.—*Composition of roots and crowns of healthy, pruned, and diseased sugar-beet plants. Calculated as percentage of the fresh weight. Greenhouse-grown, June, 1936*

Fractions	Roots			Crowns		
	Healthy	Pruned	Diseased	Healthy	Pruned	Diseased
Total solids	23.83	22.01	19.85	24.14	21.46	19.47
Soluble solids	18.18	16.71	14.80	17.22	15.12	13.92
Insoluble solids	5.65	5.30	5.05	6.92	6.34	5.25
Total sugars	15.39	13.16	12.22	13.81	12.34	11.36
Sucrose	15.27	13.03	12.08	13.63	12.26	11.18
Glucose	0.12	0.13	0.14	0.17	0.14	0.18
Fructose	0.00	0.00	0.00	0.01	0.02	0.00
Pectin	1.46	1.38	1.49	1.62	1.78	1.67
Total nitrogen	0.096	0.109	0.161	0.185	0.183	0.237
Insoluble nitrogen	0.068	0.064	0.069	0.110	0.105	0.088
Soluble nitrogen	0.028	0.045	0.092	0.075	0.078	0.149
Amide nitrogen	0.004	0.004	0.007	0.003	0.003	0.008
Amino nitrogen	0.008	0.009	0.018	0.011	0.015	0.025
Diamino and basic nitrogen	0.009	0.006	0.016	0.011	0.012	0.016
Volatile bases and ammonia nitrogen	0.004	0.006	0.015	0.008	0.008	0.026
Nitrate and nitrite nitrogen	0.005	0.007	0.007	0.005	0.007	0.011

In the course of this study it was thought that pectic materials might be more abundant in diseased than in healthy roots and, they might, therefore, interfere in the manufacturing process. Table 1, however, reveals that there was no significant difference between healthy, pruned, and diseased roots and crowns with regard to pectin content.

Total nitrogen was greater in diseased roots and crowns than in healthy and pruned. This agrees with the work of Saillard (5). Although his study was concerned with roots only, he reported that certain nitrogenous compounds were higher in beets infected with *Cercospora beticola* than in healthy sugar beets. Murphy (4), also, reported the presence of increased amounts of ammonia, amide, nitrate and nitrite nitrogen in susceptible varieties of oats infected with *Puccinia coronata avenae* Eriks., while the sugars, sucrose, glucose and levulose, showed marked decreases. In the first experiment, table 1, there was little difference between the healthy and the pruned, while in the second experiment, table 2, the nitrogen content of the pruned roots and crowns was somewhat lower than for the healthy, perhaps because of export into new leaves.

The higher total nitrogen content of roots and crowns from leaf-spot-infected plants suggests that there was a movement of nitrogenous com-

TABLE 2.—Composition of roots and crowns from healthy, pruned, and diseased sugar-beet plants. Calculated as percentage of the fresh weight. Field-grown. October, 1937

Fractions	Roots			Crowns		
	Healthy	Pruned	Diseased	Healthy	Pruned	Diseased
Total solids	20.41	19.48	17.06	20.58	20.48	16.77
Soluble solids	15.90	15.40	13.20	14.60	15.20	11.90
Insoluble solids	4.51	4.08	3.86	5.98	5.28	4.87
Total sugars	13.24	12.70	10.92	11.90	12.84	10.19
Sucrose	12.96	12.33	10.66	11.14	12.22	9.65
Glucose	0.28	0.37	0.26	0.76	0.62	0.54
Total nitrogen	0.183	0.174	0.229	0.285	0.242	0.314
Insoluble nitrogen	0.086	0.071	0.067	0.145	0.114	0.104
Soluble nitrogen	0.097	0.103	0.162	0.140	0.128	0.210
Nitrate and nitrite nitrogen	0.014	0.016	0.019	0.016	0.015	0.027

pounds from diseased leaves. This point will be referred to later under blades and petioles. The greater nitrogen content may have been the result of less growth and the same or a greater rate of absorption from the soil. The difference between healthy, pruned, and diseased roots and crowns was attributable to differences in soluble nitrogen compounds and not protein.

The soluble nitrogen compounds were fractionated in the first experiment (Table 1). From these data it is observed that the soluble nitrogen constituents followed the soluble nitrogen trend. Nitrate nitrogen was slightly higher in roots and crowns from diseased plants than in those from healthy or pruned plants. The results obtained with the following analyses: amide nitrogen, amino nitrogen, diamino and basic nitrogen, volatile bases and ammonia nitrogen, nitrate and nitrite nitrogen furnished small differences.

Leaves

Carbohydrates are manufactured by photosynthesis in the blades and move to the crowns and roots *via* the petioles. The leaf-spot organism attacks mainly the blades, or the sugar-making tissue; hence, it was considered of interest to investigate the effect of the leaf spot upon the composition of the blade and petiole and also upon the ability of the blade to carry on photosynthesis under different degrees of infection with *Cercospora beticola*.

In diseased leaf blades (Table 3) total solids, insoluble solids, and acid-hydrolyzable materials were greater than in the healthy blades. Total sugars, soluble solids, sucrose, glucose, and pectin were lower, while fructose and dextrin showed small differences.

In diseased petioles insoluble solids, dextrin and acid-hydrolyzable materials were higher, while the healthy petioles had a higher percentage of total solids, soluble solids, total sugars, sucrose, and fructose. Glucose showed only a slight difference.

The lower sugar content in diseased blades and petioles as compared with similar healthy tissues may be related to a slower rate of manufacture in

TABLE 3.—*Composition of healthy and diseased sugar-beet blades and petioles. Calculated as percentage of the dry weight. Total solids calculated as percentage of the fresh weight. Greenhouse-grown, 1937*

Fractions	Blades		Petioles	
	Healthy	Diseased	Healthy	Diseased
Total solids	15.07	17.77	11.38	9.76
Soluble solids	33.16	30.45	55.71	49.59
Insoluble solids	66.84	69.55	44.29	50.41
Total sugars	3.32	2.48	35.32	27.35
Sucrose	1.99	1.18	9.84	3.48
Glucose	1.33	1.24	21.09	21.31
Fructose	0.06	0.05	4.39	2.56
Dextrin	6.70	6.92	3.43	4.09
Acid-hydrolyzable	2.19	5.45	5.27	6.65
Pectin	7.69	6.97

diseased blades and to some of the sugar being utilized by the pathogen. Leonard (2) has shown that sugars move out of the blades of the sugar-beet plant into the petioles in a polar direction. This action probably prevents the sugars being removed from either the petioles or the root by activities of the fungus in the blade.

Experiments were conducted on diseased and healthy sugar-beet leaves taken in August to determine what influence varying amounts of leaf-spot necrosis had upon the capacity of the leaf to remove CO₂ from the air. Detached sugar-beet leaves from field-grown plants were used, since no greenhouse plants were available that possessed a sufficient number of classes of diseased leaves to provide the desired range. Some tests, however, were run on potted greenhouse plants to confirm the results obtained with the detached field-grown leaves.

It is clear from the data in table 4 that the capacity of a leaf to remove CO₂ from the air decreases as the leaf spotting increases. Most of the photosynthetic values were obtained at a light intensity from 2000–4000 foot candles with air temperature of 20°–24° C. The respiration values were taken at night. The results presented in table 4 are averages from 10 replications. The condition of the leaves was normal turgidity. The necrotic areas were fully developed and dry, in which case the pathogen, or at least that portion of it within the necrotic area or areas, may be considered as being nearly inactive and its respiration at a minimum. In view of this condition a class 6 leaf injury, one in which the leaf is entirely killed by the pathogen, was placed in a moist atmosphere for 2 hours preceding the recording of respiration, the results of which appear in table 4.

The leaf-spot fungus, and also any saprophytic organisms that might have been present, became more active after the leaves had taken up considerable moisture as the result of having been placed in a moist chamber for 2 hours. The evolved CO₂ increased 3 times its dry rate. Excessive quantities of CO₂ released by the pathogen were not responsible for the lowered rates of apparent photosynthesis obtained in the case of the various classes of diseased leaves.

TABLE 4.—*Photosynthesis and respiration in detached healthy and diseased sugar-beet leaves. Presented as mg. CO₂/100 sq. cm./hour. Field grown, 1938.*

Leaf classes Amount of leaf-spot necrosis	Photosynthesis	Respiration
Healthy (check)	6.30	0.79
Class 2	5.39
Class 3	3.21	0.61
Class 4	1.14	0.86
Class 5	0.49	0.50
Class 6 ^a	0.69
Class 6 ^b	1.95

^a Only midrib remained green, pathogen induced complete necrosis of blade.

^b Leaves held 2 hours in a saturated atmosphere.

The results obtained with leaves detached from plants grown in the field were in part verified by attached leaves on greenhouse-grown plants. The diseased plants grown in the greenhouse did not possess all the classes of diseased leaves, hence only the following data were obtained: healthy leaves, 6.47 mg. CO₂/100 sq. cm./hour; class 2 leaves, 5.70 mg. CO₂/100 sq. cm./hour; class 3 leaves, 4.70 mg. CO₂/100 sq. cm./hour, with a light value of 2000–4000 foot candles. These results closely approximate those obtained with field-grown material.

The crowns and roots of diseased sugar beets are higher in total nitrogen than the corresponding healthy tissues. It would be interesting to know whether the increased nitrogen in these tissues is due to increased absorption, decreased growth, or the transport from leaf-spot-infected leaves. To test the latter point, blades and petioles consisting of the various classes of necrotic leaves were analyzed for total nitrogen.

TABLE 5.—*The percentages of total nitrogen in healthy sugar-beet leaves compared with leaves possessing varying amounts of Cercospora leaf-spot necrosis. Field grown, 1938*

Leaf classes Amount leaf-spot necrosis	Total dry weight Percentage of fresh weight	Total nitrogen Percentage of dry weight
Healthy (check)		
Blades	14.09	5.63
Petioles	7.20	2.54
Class 3		
Blades	19.43	4.80
Petioles	7.32	2.28
Class 4		
Blades	24.30	4.47
Petioles	7.69	2.32
Class 6		
Blades	4.02
Petioles	7.20	2.25

It is evident from the data in table 5 that there was a decrease in total nitrogen in the diseased blades. Probably the nitrogen moved to the roots and was at least partially responsible for the higher nitrogen content of the

diseased roots. It is probable also that part of the nitrogen was leached from the dead blade tissues by dew and rain.

SUMMARY

Diseased, pruned, and healthy sugar-beet plants were compared as to composition in an effort to determine the effect of the leaf-spot organism upon the sugar-beet plant.

Cercospora leaf spot reduced the percentage sucrose in the crowns and roots as well as in petioles and blades.

Total nitrogen in diseased roots and crowns was greater than in similar healthy and pruned tissues. In the field-grown plants healthy and pruned roots did not differ greatly in composition, and there was little difference between healthy and pruned crowns. There was, however, considerable variation in greenhouse material, with the exception that little difference in the pectin content was found in diseased and healthy roots.

As leaves became more severely affected with leaf spot, their ability to assimilate CO₂ from the air diminished.

Roots and crowns from diseased plants were higher in soluble nitrogen than similar healthy and pruned tissues. Analysis of diseased and healthy leaves showed that the percentage of total nitrogen decreased with the severity of infection. This decrease may have been caused by translocation from dying tissues into the crowns and roots.

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THE THIXOTROPIC CHARACTER OF THE TOBACCO-MOSAIC VIRUS PROTEIN

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A certain degree of progress in the study of the plant viruses is attributable to the use of techniques used in some phases of protein chemistry. In the enthusiasm attendant on the occasion of this progress, interpretations have been proposed and assertions made that, in the light of further evidence, may well be subject to question. Although it has been stated (12) that "the chemist, after a perusal of the physical and chemical properties of

the tobacco-mosaic virus protein, has no difficulty whatsoever in coming to the conclusion that, despite its huge size, it has *all* the properties of a molecule," lack of characteristic molecular properties has now been demonstrated.¹ Some of the difficulties standing in the way of an acceptance by the physical chemist of interpretations based on the assumption that the protein-water system is a solution of molecularly dispersed supermolecules are presented in this paper.

There are many systems known that have the property of changing from a gel to a fluid state on mechanical disturbances, and spontaneously reverting to a gel state after the fluid has come to rest. The rigidity of the gel, and the time of transition from the liquid to the gel state, will vary from system to system. By the way of an example, one may prepare a suspension of bentonite in water, which, if permitted to stand for a few minutes, will set to a gel that will retain its form even if the beaker be inverted. If the system be agitated the gel properties will vanish and the system will flow (superficially) like water, but the structure, broken by forced flow, will spontaneously reform. Systems of this type are said to be thixotropic. Use is made of this phenomenon in drilling operations in deep oil wells. A "drilling fluid" (that is a thixotropic sol) is added during the drilling. So long as the bit is in operation the fluid is mobile. If the drilling is suspended, the fluid will set to a gel, retaining the rock fragments in suspension; otherwise, they would settle to form a compact mass about the bit and might make resumption of the drilling impossible.

A characteristic property of these systems is that the suspended particles do not exhibit Brownian movement, but rather they are held rigidly in position by forces of electrostatic nature. These quiescent systems form an elastic structure, and, so far as is known, all elastic systems obey Hooke's law so long as the elastic limits are not exceeded.

Diffusion phenomena, osmotic pressure, freezing-point depressions, boiling-point elevations, and other related phenomena, are, in reality, manifestations of thermal motion or Brownian movement. In systems where the thermal motion is restricted because of interparticle forces, the various laws quantitatively relating the various phenomena listed above with the the number of suspended particles per unit volume of solution are no longer applicable. Stoke's law, Fick's law, Raoult's law, and others, are not followed, and they may *not* be relied upon to give one a true estimate of the size of suspended particles (which, incidentally, are not necessarily molecules). In general, the viscosity of these systems is anomalous, and there is never agreement in the size of the particle, as determined by various independent means.

The tobacco-mosaic virus protein shows anomalies in diffusion and viscosity (4, 5, 6, 10). The viscosity in particular is typical of that shown by thixotropic systems. In the case of the virus protein, the thixotropic gel is not sufficiently rigid to withstand the inversion of the container, but

¹ *Italic is mine.*

it is possible to demonstrate the gel nature of the quiescent water-protein system by other means. The observations reported were made using a protein preparation obtained in the usual manner, that had been electro-dialyzed, and subsequently dispersed in distilled water. The photographs were taken on 16 mm. Kodachrome movie film, and in some instances they were taken through crossed polaroid plates.

It has been reported (4) that a sphere dropped through a virus-protein sol will leave a trail in its wake—a trail that may be observed through crossed polaroid plates—that will persist for a considerable period of time. Figure 1, A, shows such a trail. In this particular case, the sphere was a cabbage seed falling through an .87 per cent sol. Trails formed at room temperature and under conditions that do not subject the gel to undue disturbances will persist upwards of 24 hours. Bawden (1) reports that the upper layer, formed by permitting a 1.6 per cent sol to stand, will show anisotropy of flow that persists for a long time after flow has ceased. The interpretation is that the sol-gel transformation time is short, and that the orientation of the anisodimensional protein aggregates induced by flow of the sol around the falling sphere persists because of the restriction of Brownian movement.

The behavior of vortices in the water-protein system are of particular interest. Vortex motion in a fluid may be induced by sudden disturbances as, for example, the blowing of smoke rings by an abrupt movement of the tongue. The vortex lines either form closed curves (rings) or they are bounded by the surface of the fluid, and the vortex always consists of the same fluid particles. In the case of a frictionless fluid, the strength of the vortex is constant in time. In actual cases, however, where the fluid has a definite viscosity, the vortices are gradually dissipated. Nevertheless, in fluids having viscosities as great as ordinary aqueous solutions, typical vortices, formed by a drop of the solution falling into the surface through 2 or 3 mm., will move through the liquid for a considerable distance and at a fairly rapid rate of speed. Easily visible vortices may be formed by dropping a dilute solution of AgNO_3 into a dilute solution of NaCl . A vortex formed in this manner is shown in figure 1, B. A vertical view is shown in figure 1, C. The feathery-shaped precipitate shown above the vortex in the one case, and in the center in the other, are due to secondary disturbances attributed to the splash. Vortices comparable to these are not formed in sols of the virus protein when a drop of the sol falls into the surface of the protein sol. By making observations through crossed polaroid plates, one may see that the vortex so formed does not proceed rapidly through the protein-water system, but rather will set, as it were, retaining its form only a few millimeters below the surface. A vertical view of several "frozen" vortices is shown in figure 1, D.

On the other hand, a moderately concentrated solution of BaCl_2 dropped into a sol of the virus protein (1.6 per cent) containing a small amount of ammonium sulphate in solution will behave in the manner depicted in

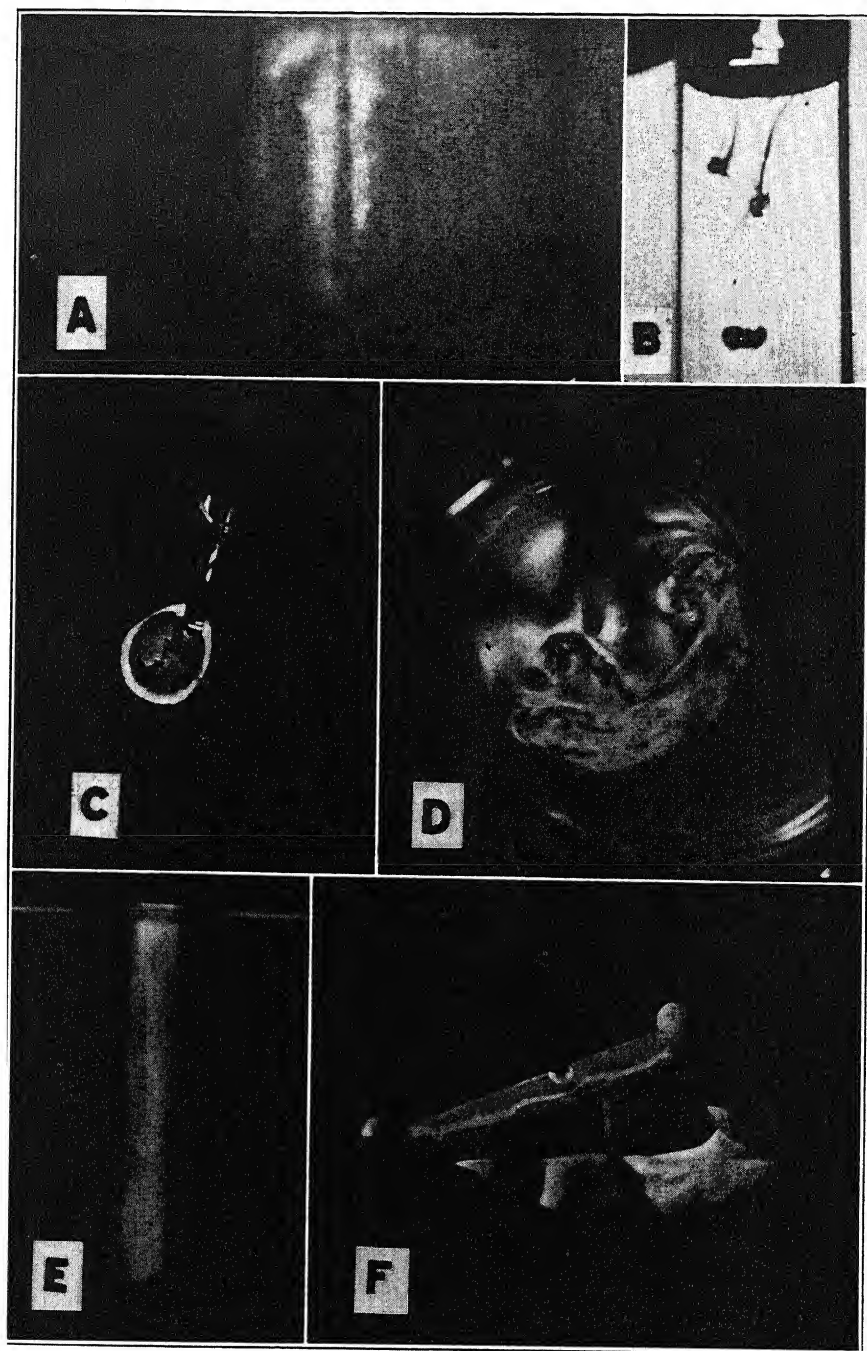


FIG. 1. See text for legend.

figure 1, E. Some concept of the rigidity of the precipitated BaSO_4 "rod" may be obtained by inverting the test tube. In figure 1, F, the bubble of air trapped at the top of the tube did not disturb the precipitated salt on flowing up the inverted tube, that is from the index finger at the mouth of the tube to the thumb at the base.

The rigidity of the thixotropic gel may be further demonstrated by drawing a line through the gel by means of a glass rod. The line may be seen through crossed polaroid plates, and it will remain at rest relative to the beaker as the beaker is rotated about an axis normal to the gel surface. That is, the system behaves as a solid. It is well known, for example, that in the case of a true liquid the fluid does not rotate with the slowly rotating container, but, because of its inertia, will remain at rest relative to stationary objects. Flow occurs along the walls of the container. In the case of the virus-protein gel, oscillation may be induced in the gel by abruptly turning the beaker through a small angle. A line defined by a glass rod drawn through an .8 per cent sol is shown in figure 2, and the photograph shows the rod half way across the beaker, drawn at right angles to the original path. As in the case of the trail left by the falling sphere, these lines will persist for hours.

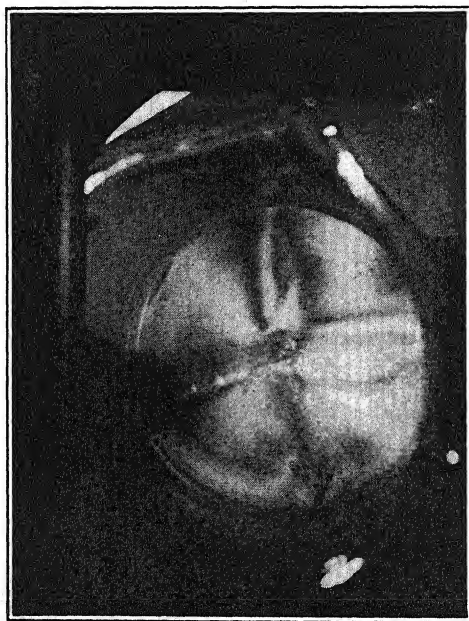


FIG. 2. Lines through the thixotropic gel defined by a rod drawn through the quiescent system. A cross is in the process of being completed. Photographed through crossed polaroid plates.

Possibly better evidence for the spontaneous repair of structure broken by forced flow may be obtained by inducing flow in an .8 per cent sol by giving the beaker a rotatory motion. The velocity of the sol decreases

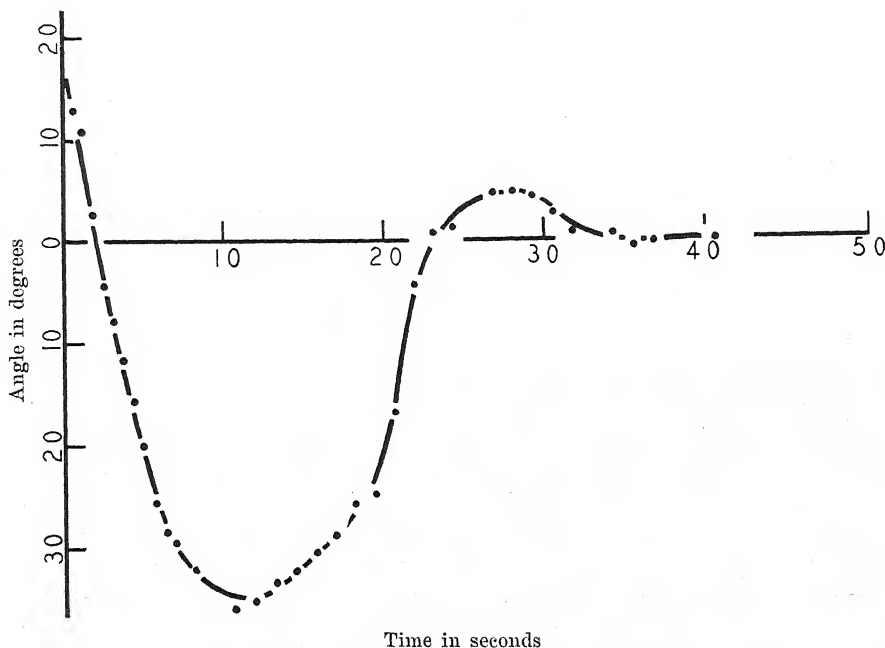


FIG. 3. Strongly damped harmonic motion described by the thixotropic gel immediately after the spontaneous repair of the structure that had been broken by forced flow. Abscissa is in seconds; ordinate is angle in degrees formed at the intersection of a line joining the center of the cross of isocline and a suspended debris particle at the time t and a line joining the center of the cross of isocline and the same debris particle after the oscillation had died down.

rapidly after the beaker is set at rest, stops abruptly, and then oscillates about an axis normal to the surface. The behavior is shown in figure 3. The data were taken from motion pictures of the sol that had been set in motion in the manner indicated above, and in which several particles of debris has been suspended. The film was projected onto cross-section paper with the coordinates taken parallel to the edges of the picture, and the origin as the center of the cross of isocline. The coordinates of the particle positions in successive frames were recorded, and the curve given in figure 3 was constructed on the basis of these data. The time interval between successive frames was assumed to be $\frac{1}{16}$ second. The abscissa is in seconds, and the ordinate is the angle in degrees formed at the intersection of the line joining the center of the cross of isocline and a suspended debris particle and the line joining the center of the cross of isocline and the same debris particle after the oscillation had died down.

The behavior of the oscillating gel mass is reminiscent of that shown by a pendulum oscillating in a viscous medium. The strongly damped harmonic motion is well known in physics, and is quantitatively accounted for by supplementing Hooke's law with a retarding force that is proportional to the velocity of the oscillating object. According to Hooke's law, displacement in an elastic medium is opposed by a force of restitution that

is proportional to the extent of the displacement. A test for monodispersity in the ultracentrifugal work is the linearity between $\log C$ and X^2 where C is the concentration at the point X and X is the distance from the center of the centrifuge. Such a linear relationship would be characteristic of a gel or any elastic system that obeyed Hooke's law (13). These points are emphasized because of the recorded statements relative to the homogeneity of the virus protein, and the various interpretations based on these statements that have been advanced. It probably would not be amiss to add that a great deal of care is taken to assure quiescence of the various systems in the diffusion work and in the ultracentrifugal studies.

A further significant consideration is the failure of the protein to yield saturated "solutions" in water. There is no discontinuity in the physical properties as the system passes from the moist protein mass collected at the bottom of a centrifuge tube to the so-called solution state. As one adds water, the protein aggregates merely separate, with the degree of separation depending on the attendant change in volume; the reduction in the rigidity of the thixotropic gel increases correspondingly. The system merely swells. There is no equilibrium between Stanley's "crystals" and the solution.

A few additional remarks may be advanced in further discussion. The velocity profile of a viscous fluid flowing through a capillary tube is a parabola. In the center of the capillary the velocity of one fluid layer relative to a second adjacent layer is not great. Considering, then, the capacity of the virus protein sol to spontaneously gel, even while under substantial shearing stresses, it is obvious that the flow through the capillary must be abnormal. It has been demonstrated (3) that for systems of this kind, the velocity profile in a capillary is that of a truncated parabola—the material going through the center of the tube acts as a rod. It has not been possible to determine the true viscosity of these systems, hence we may not accept as reliable such calculations as are made on the assumption that one has measured viscosity. Thus the criticisms that were directed by Robinson (11) against the work of Lauffer (7, 8, 9) are valid criticisms, but they are applicable to his own interpretations with an equal validity; viscosity data cannot be used for ascertaining the ratio of length to thickness of the virus particles because of the thixotropic character of these pseudo-solutions.

Perhaps one should not dismiss the question of the ratio of length to thickness of the virus protein aggregates without some attention to the suggestion of Bawden and Pirie (2). They assumed that the critical concentration at which a solution becomes spontaneously birefringent is determined by the length of the rods, and that at the critical concentration the ratio of the volume occupied by the virus protein to that occupied by the water should equal the ratio of the volume of the rod to the volume in which the rod can rotate freely. The effects are presumed to be mechanical. On the basis of these assumptions, and the observation that the sol becomes spontaneously birefringent at a concentration of 1.6 per cent, they calculated a ratio of thickness to length of 1:90. It is pertinent to point out in this con-

nection that thixotropic behavior has not been accounted for satisfactorily on the basis of any simplifying assumption. Thixotropic behavior cannot be accounted for on the basis of a simple mechanical interference, and the effects are ascribed to imperfectly understood forces acting through a distance.

Thus, in summary, the tobacco-mosaic virus protein particles in "solution" not only fail to show all the properties of molecules, they fail to show the most characteristic property of molecules in solution, namely, that of unrestricted thermal motion.

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EVIDENCE OF PASSIVE IMMUNIZATION OF TOBACCO, NICOTIANA TABACUM, FROM THE VIRUS OF CURLY TOP

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It has been shown in a number of instances that plants infected with one virus are unaffected by subsequent inoculations with certain other viruses. It has been reported also that recovery of plants from viroses is sometimes accompanied by an acquired immunity.

Wingard (8) demonstrated that, following infection with the ring-spot virus, tobacco plants recovered by producing new growth less severely

affected, or apparently devoid of symptoms, and that the recovered plants were unaffected by reinoculations with ring-spot virus. It was further shown that the recovered plants contained virus capable of producing typical ring-spot symptoms when used for inoculation of healthy plants. Price (5) corroborated Wingard's findings and showed that virus was still present in plants after 10 serial cuttings.

Johnson (3) reported that plants of *Nicotiana tabacum*, *N. sylvestris*, and *N. repanda*, recovered from the necrotic stage of tobacco streak and thereafter failed to develop this stage of the disease when reinoculated with streak virus.

Lesley and Wallace (4) found that plants of certain wild races of tomato often partially recovered from curly top and that such plants were unaffected by subsequent inoculations with curly-top virus. In this instance, too, the recovered plants still contained virus unchanged in virulence. Wallace (6) reported a similar behavior of *Nicotiana tabacum* plants following partial recovery from curly top.

The reactions described above have been designated by certain authors as "acquired immunity," but this interpretation does not find complete acceptance. With our present limited knowledge of the nature of these plant-virus reactions, some workers believe that the term "acquired tolerance" is preferable for designating this phenomenon in plants. This diversity of opinion results, it seems, from individual differences in the definition of the term "acquired immunity," and further, from different viewpoints as to whether the plant reactions are sufficiently similar to the known immunological reactions in animals to warrant designating the plant reactions as "acquired immunity." Although a conformity of terminology is desired in this connection, it will probably not be attained until the nature of the plant reactions is better understood. The studies reported in this paper seem to provide in part, at least, needed evidence for a further understanding of these plant-virus reactions. In view of this evidence, the term "acquired immunity" is used in this report. As used here, "acquired immunity" denotes a condition in which recovered plants show a high degree of tolerance of the virus remaining in them, and are highly resistant to reinoculation with the same virus or with a closely related strain. In the following portion of this paper other similarities between these plant-virus reactions and acquired immunity as known in animal studies will be presented.

The question of whether plants can develop antibodies or protective substances has been a subject of both investigation and speculation over a long period of years. The apparent immunological behavior of certain plants that recover from viroses is strongly suggestive of some such reaction. While a demonstration of protective substances may not be required to establish proof of acquired immunity in plants, such evidence would aid in establishing a homology between immune reactions in plants on the one hand and those of animals on the other. Serological evidence of virus antibodies in

plants would no doubt be accepted as proof of the similarity of immune-reactions in plants and animals, but such evidence yet remains to be obtained. If, however, it can be shown that, as a result of a disease process, protective or reaction-lessening substances are produced in plants and that these, in turn, can be transferred to healthy plants, then an approach will have been made towards results more comparable with the classic types of active and passive immunity.

A number of investigators working with fungous and bacterial diseases have attempted to transfer natural resistance from resistant to susceptible plants by grafting, and similarly to transfer susceptibility to resistant plants. Generally, these efforts gave negative results, with no indication of cion-stock influence on disease reaction. Price (5) attempted to transfer what he concluded to be an active immunity in tobacco plants, recovered from ring spot, by grafting these plants with healthy ones. Typical ring-spot symptoms always appeared on the healthy plants. Price, therefore, concluded that there was no transfer of protective substances or antibodies from recovered to healthy plants. On the other hand, Wallace (7) found that the acquired immunity of Turkish tobacco plants that recovered from curly top could be transmitted from such plants to healthy plants by grafting and it is the purpose of the present paper to report further details of this study.

Turkish tobacco plants that recovered from curly top were frequently almost normal in appearance but usually showed mild symptoms. When cions from recovered plants were grafted on healthy plants, the healthy stocks became diseased but developed mild symptoms. Likewise, cions from healthy plants developed mild symptoms when grafted on recovered plants. Reciprocal grafts of various kinds, and approach grafts in which both recovered and healthy plants grew on their own roots, likewise resulted in the production of mild symptoms on the previously healthy parts. The severity of the initial symptoms on healthy parts infected from recovered plants was influenced, to some extent, by the treatment of the healthy plant or plant parts. If the terminal of the graft-infected parts was removed at the time of grafting, the new growth from axillary buds, particularly the uppermost bud, frequently showed severe symptoms in the early stages of growth. However, the prompt resumption of growth of such shoots, accompanied by progressively less severe symptoms was strikingly different from the behavior of axillary shoots that developed on plants infected by leaf-hopper inoculation and similarly cut back during the early stages of infection.

Reinoculation of plants that became mildly diseased after grafting with recovered plants showed that such plants had acquired the same degree of resistance as has been reported previously for plants propagated vegetatively from tobacco plants that had recovered from curly top (6).

The results of this study suggested that the recovered plants contained protective substances that moved with the virus through the graft unions

and protected the newly infected tissues from severe injury. If such substances were not responsible there seemed to be only two other possible explanations. One of these was that the virus in recovered plants had become attenuated, or so changed in virulence that it would produce only mild symptoms when introduced into healthy plants. The second possible explanation was that infection through graft unions might in some way affect the resulting severity.

If the virus in the recovered plants were attenuated it would be expected to produce mild symptoms on healthy plants infected either by grafting with the recovered plants or by leaf hoppers that acquired virus from the recovered plants. Direct transfer of virus from recovered plants to healthy plants by means of leaf hoppers resulted in typical, severe symptoms; and this is accepted as proof that the virus was not attenuated in the recovered plants. Over a period of several years, extensive study of strains of curly-top virus has given no evidence that virulence is affected in any respect by passage through the insect vector.¹ In the work of Giddings (2), 4 different curly-top strains were repeatedly transferred by individual leaf hoppers in experiments involving hundreds of inoculations, and there was no evidence of change of virulence of the virus by the insects. Therefore, it is not believed that attenuation of virus in the recovered plants was responsible for the results obtained in the present study. To investigate the effect of infection through graft unions on severity, healthy cions were grafted on healthy stocks. In some cases the stocks, in others the cions, were inoculated by leaf hoppers. Also, pairs of healthy plants were approach-grafted and one member of the pair was inoculated. Typically severe symptoms usually developed on both the inoculated and noninoculated members of the graft pair. This proved that the virus could be introduced on one side of a graft and that it passed through to the other side and produced symptoms there as severe as those that developed on the parts infected directly by leaf hoppers.

With the evidence at hand strongly suggesting that recovered plants contained something of antibody-like function, other experiments were conducted to determine if healthy plants infected by grafting with diseased plants, not showing recovery, would become more severely diseased than those infected by grafting with recovered plants. If the healthy plants grafted with recovered plants developed mild symptoms because they received protective substances from the recovered plants, then healthy plants grafted with diseased, nonrecovered plants should develop typical, severe symptoms because of their not receiving protective substances. In early experiments of this kind it became evident that in leaf-hopper-inoculated plants, the reaction leading to recovery took place at an early stage following infection, and that it was completed or at least well-advanced before the plants began to recover. Such striking differences were obtained,

¹ Unpublished data. Division of Sugar Plant Investigations, U. S. Dept. of Agri. Field Station, Riverside, California.

depending on the period over which the virus had been present in the leaf-hopper-inoculated cion plants, more extensive experiments were immediately undertaken. Healthy tobacco plants were grafted with cions taken from leaf-hopper-inoculated plants at 5-day intervals up to 25 days following inoculation. Plants from which cions were to be taken were inoculated by caging viruliferous leaf hoppers on the terminal leaves of each plant. After the desired period of time following inoculation, the terminals were removed and grafted laterally to healthy tobacco plants. Usually about 7 days are required for phloem connections to be formed between cion and stock in *Nicotiana tabacum* (1). Thus, no transfer of virus or probably any other phloem materials can take place until these connections are established. If a development of protective substances begins when the plants are inoculated, the process very probably continues in the terminals during the interim of grafting and the time of development of phloem connection. If so, in a cion removed from an inoculated plant on the 10th day following inoculation and grafted to a healthy plant, there would be a total period of about 17 days for the production of protective substances before these substances and virus from the cion could move into the healthy stock plant.

In one typical experiment, healthy plants were grafted with 5-inch terminal cions taken from plants inoculated 5, 10, 15 and 20 days previously. At time of grafting, the plants inoculated 5 days previously showed no curly-top symptoms, those inoculated 10 and 15 days previously showed symptoms of moderate severity, and the plants that had been infected 20 days were severely diseased. The results were clear-cut. Healthy plants developed severe symptoms when grafted with cions taken from inoculated plants on the 5th day after inoculation. Those grafted with cions from plants that had been inoculated 10 and 15 days previously, developed symptoms of an intermediate severity, that is, severe for a period of time, but gradually becoming mild. Healthy plants infected from cions from plants in which curly-top virus was operative for 20 days previous to grafting, developed mild symptoms. Figure 1 shows the reactions of healthy stock plants after grafting with cions taken from inoculated plants at different periods following inoculation. The cion on plant 1 was a severely diseased terminal of a plant inoculated by leaf hoppers 20 days prior to removal of the cion for grafting. The cion on plant 2 was taken from an inoculated plant on the 5th day after inoculation, and showed no curly-top symptoms when the graft was made. The stock plants were of the same age and size and were grafted at the same time. The reactions of the plants shown in figure 1 are typical of results obtained when experiments of this kind were conducted under conditions generally favorable for curly-top development. Environmental conditions were found to influence, to some degree, results obtained in this type of test. When conducted at temperatures low enough to retard symptom development, all of the graft-infected stock plants were mildly affected. It was concluded that factors that retarded virus multipli-

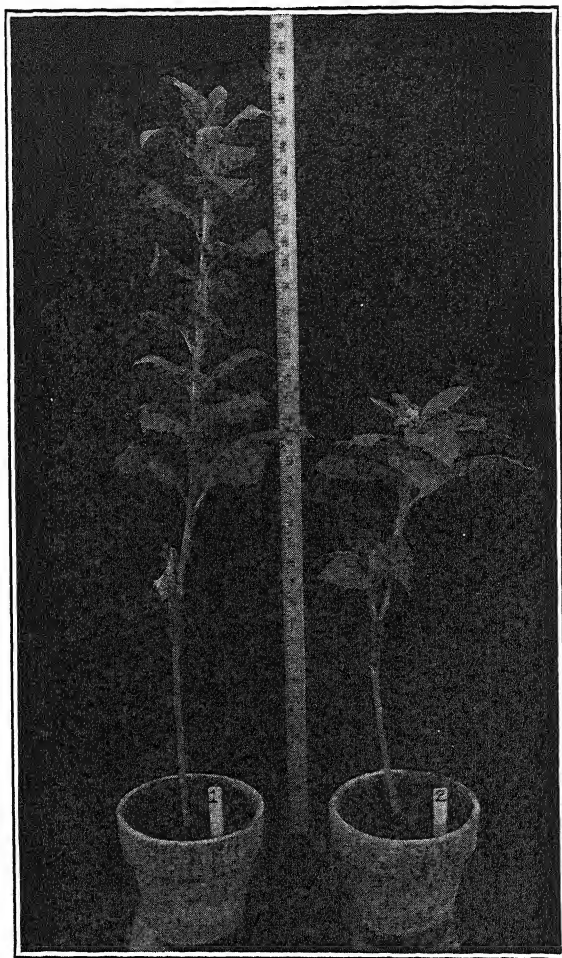


FIG. 1. Reactions of healthy stock plants when grafted with cions taken from leaf-hopper-inoculated plants having different periods of time for development of protective substances. 1. Mild symptoms on stock infected from cion in which curly-top virus had been operative 20 days prior to grafting. 2. Severe symptoms, stunting of plant and severe curling of top leaves, on stock infected from cion taken from a plant inoculated 5 days prior to grafting.

cation and activity enabled the recovery, or protective reaction to take place before the virus produced its maximum effect on the plants.

Further details of this study will appear later. The data presented in this paper seem to offer strong evidence that Turkish tobacco plants infected with curly-top virus are capable of developing protective substances whereby the plants are enabled to recover and tolerate the virus remaining in the plants. Plants so protected can not be reinfected, that is, they are apparently immunized, and most important in indicating that this protective substance exists is the evidence that it can be transferred to other plants where it serves to lessen severity of curly top and hastens recovery. In short,

the phenomena are comparable to those known in animal diseases in cases of active immunization and the production of passive immunity by protective substances or antibodies. On the basis of the evidence obtained in these experiments the behavior of the recovered plants is interpreted as acquired immunity and that this acquired condition results from protective substances produced by a specific reaction between the tobacco plants and the curly-top virus. Since plants in which this reaction has occurred retain virus unaltered in virulence, as shown by insect vector transmission to healthy plants, the production of mild symptoms on healthy plants grafted with plants having this acquired immunity is believed to be an example of a kind of passive immunization, a phenomenon not previously reported in plants.

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THE WINTER CARRY-OVER OF ANGULAR LEAF SPOT INFECTION IN ARIZONA COTTON FIELDS

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(Accepted for publication February 26, 1940)

INTRODUCTION

The cotton disease known as angular leaf spot, bacterial blight, or black arm, caused by the *Phytophthora malvacearum* E.F.S., is generally believed to be transmitted almost entirely on the seed. Most investigators have recognized the possibility of its persistence in nature, and workers in Egypt¹ have reported evidence of its carry-over on plant remains from the previous crop. In the cotton-producing areas of Arizona, the mild winters and short interval between successive cotton crops seem to favor winter carry-over. The commonly used methods of disinfecting the planting seed with organic mercury compounds or of delinting with sulphuric acid usually are effective

¹ Massey, R. E. Studies on black arm disease of cotton. III. Empire Cotton Growing Rev. 11: 188-193. 1934.

in controlling the disease in the seedling stage, but in some cases fields that show no infection on the planted seedlings when they first emerge, later show widespread infection. This infection may occur during the seedling stage, or it may be delayed until the rainy period, which occurs usually in late summer.

King and Parker,² in 1939, reported evidence that infection on volunteer seedlings may be the source of disease that is spread to the planted crop by irrigation water. In 1939 the writers continued the study of winter carry-over and attempted to determine the extent to which seed cotton remaining in the fields might be responsible for transmitting infection to the succeeding crop. The results of the study are reported in this paper.

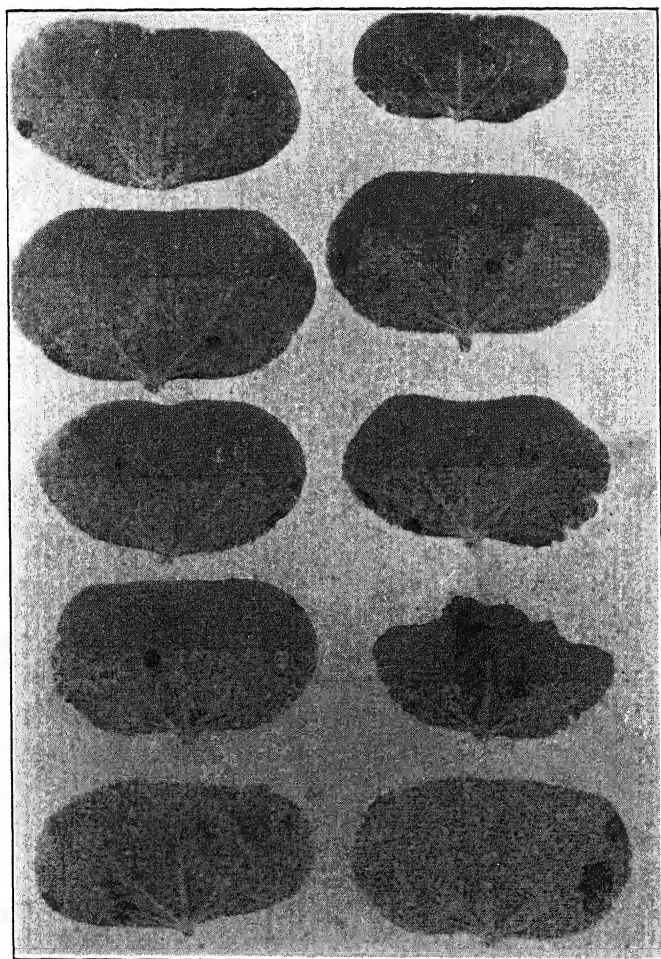


FIG. 1. Angular leaf spot lesions on the cotyledons of Acala cotton seedlings grown in greenhouse from seed that had overwintered in the field.

² King, C. J., and R. B. Parker. Angular leaf spot of cotton in irrigated valleys of Arizona and New Mexico. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 23: 32. 1939.

GREENHOUSE TEST OF FIELD EXPOSED SEED

On February 25 and March 30, 1939, some samples of seed cotton of the Acala variety were collected from a field near Eloy, Arizona, that had shown severe infection the previous season. One lot was collected from the old burs of plants that were still standing and on which black-arm lesions on the main stem were still apparent; another lot was gathered from the ground beneath plants that showed black-arm lesions. The last-mentioned lot contained many exposed locks or unopened bolls that appeared to have been in long contact with soil moistened intermittently by winter rains. The samples were ginned and the seed was immediately planted in the greenhouse in large flats containing a field soil that had been clean-fallowed for 3 years. As controls, other flats were planted with Acala seed obtained from a disease-free field near Buckeye, Arizona. Infection counts were made at the time when the seedlings had developed 2 true leaves. Infection was limited largely to the cotyledons until the plants were several weeks old (Fig. 1). The results of the infection counts are given in table 1.

TABLE 1.—*Angular leaf spot infection of Acala cotton seedlings from seed that had remained in the field during the winter 1939^a*

Seed lot	No. seeds planted (approximately)	No. seedlings emerged	Percentage seedlings infected
Seed lot No. 1 Collected from old stalks	300	224	34.4
Seed lot No. 2 Collected from ground	200	97	30.0
Seed lot No. 3 (Control) Collected from disease-free field	500	423	0.0

^a On March 23, 1940, a planting was made in the greenhouse of 1600 seeds of S×P cotton seed collected March 21 after overwintering on old diseased plants in a field near Marana, Arizona. Only 110 seedlings emerged and 46 per cent of these showed angular leaf spot infection on the cotyledon leaves. A sample of 810 seeds of S×P collected March 14 from old diseased plants recently plowed under in a field near Eloy, Arizona, yielded 50 seedlings of which 34 per cent was infected.

The winter period, November 1, 1938, to February 28, 1939, as judged by the records at Sacaton, Arizona, had less than average rainfall, the total amounting to only 2.13 inches compared to a 26-year average of 3.74 inches. The minimum temperatures, however, were lower than average, with November and February mean minima being about 7° below average, while those for December and January were close to the average. The average mean minima for the 4 months, November to February, during a 26-year period were 40, 34, 34, and 38 degrees, respectively.

FIELD CENSUS OF VOLUNTEER SEEDLINGS INFECTED WITH
ANGULAR LEAF SPOT

To supplement the test of seed planted under greenhouse conditions, a census was made of the number of infected and noninfected volunteer seed-

lings that appeared in 5 fields in the same vicinity. The count was made about 10 days after planting. Usually 5 rows were selected at random in each field and on each of these sections approximately 100 yards in length were marked off. The rows being 42 inches wide, all volunteer seedlings that occurred within the strip, 21 inches at each side of the drilled seedling row, were included in the count.

Each volunteer seedling was inspected for angular leaf spot lesions, and the proportion of infected plants recorded. Many of the seedlings occurred in clusters on account of the germination of the seed from entire bolls, covered by soil in preparing the seed bed (Fig. 2). A large proportion examined in these fields showed angular leaf spot lesions soon after the cotyledons unfurled.

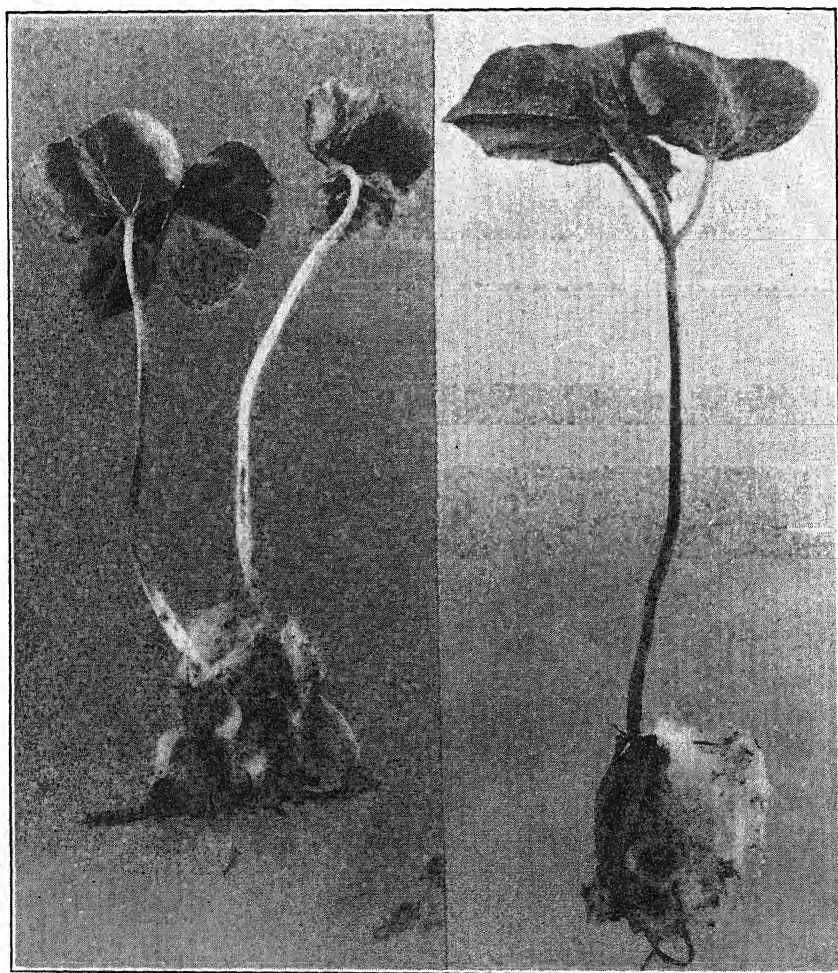


FIG. 2. Angular leaf spot lesions on Acala cotton seedlings developed from infected bolls of the previous crop plowed under in the spring.

TABLE 2.—*Number of volunteer cotton seedlings infected with angular leaf spot disease in commercial cotton fields and the proportion of the planted crop showing late-season infection in the same fields^a*

Field No.	Average No. volunteer seedlings per 100 yds. of row	Average No. infected seedlings per 100 yds. of row	Percentage of volunteer seedlings infected	Percentage of drilled seedlings infected 10 days after planting	Percentage of drilled plants on Sept. 23 with infected Leaves Stems Bolls
1	23.2	2.2	9.3	0	100 52 11
2	30.0	17.1	57.0	0	31 8 2
3 ^{ab}	16.2	2.7	16.6	11	100 33 10
4	35.0	12.4	35.7	0	28 15 3
5	36.0	5.4	15.0	0	100 83 25

^a From examination of 100 plants on same rows as those on which original counts were made or nearby.

^b Same field from which seed was collected for greenhouse test.

On September 23, the 5 fields were again visited and the plants inspected for angular leaf spot infection. The plants in most of the fields were badly lodged and it was not practicable to locate with exactness the original row sections. However an examination was made of 100 plants selected at random on or near the same rows previously inspected. The data obtained from the April and September counts are given in table 2.

Angular leaf spot infection in this district had been severe in the 3 successive crops before 1939. The extent of damage observed in September, 1939, was far less severe than that observed in the same field during September in the 3 previous years.

CONCLUSIONS

Under Arizona conditions the bacterium causing angular leaf spot may survive the winter on the seed cotton left unpicked in the fields. Since many of the seed remain viable, the volunteer seedlings developed in the spring may become a source of infection for the planted crop regardless of precautions to plant disease-free seed.

The infection on the volunteer seedling may be transmitted to the planted crop by means of irrigation water or by wind-driven rains. Some practical suggestions for minimizing the development of volunteer seedlings are: turning cattle on the fields after the cotton crop is harvested, raking and burning the plant remains, and early cultivations to destroy the volunteers before irrigating.

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THE HISTORY OF TOBACCO DOWNY MILDEW IN THE UNITED STATES IN RELATION TO WEATHER CONDITIONS

NEIL E. STEVENS AND JOHN C. AYRES

(Accepted for publication March 1, 1940)

Although the problem of endemism of *Peronospora tabacina* Adam is probably one of those for which we may never reach a solution satisfactory to all interested, it should still be worth-while to reexamine any evidence that seems to bear on this once vexed and still debated question. As is well known, this fungus was discovered in seed beds of the shade-tobacco area around Quincy, Gadsden County, Florida, and in nearby tobacco-growing regions of Georgia in the spring of 1921. Contrary to expectations the disease did little damage at that time, and was not reported in this country again for 10 years. In 1931, however, the fungus reappeared in these regions and later spread throughout the flue-cured-tobacco areas of Georgia, North Carolina, and Virginia, finally reaching southern Maryland. Since that time it has occurred in greater or less abundance every year.

As is well known, coincident with its discovery in 1921, there arose two conflicting theories to account for its appearance. One was that it had been introduced recently, probably from Australia, where it had been known since 1890. The other was that it had migrated from the western United States and had only recently become abundant in the southeast. Similarly, following its reappearance in 1931, two explanations were offered. One was that the fungus had failed to become established in 1921 and was reintroduced in 1931, the other that the fungus had been present in the region all the time, possibly on another host, but only in 1931 had there occurred conditions suitable for its epidemic development.

The case for possible endemism rested chiefly on 3 collections of downy mildews on species of tobacco, the first by Farlow on *Nicotiana glauca* in southern California, in 1885; the second on cultivated tobacco seedlings in Texas in 1906; and the third on *N. bigelovii* in Nevada, in 1914. Wolf,¹ who has examined the specimens, states that, in all 3 of these collections, oospores are lacking and that none of them can with certainty be specifically identified. He concludes "There appears no reason for regarding them as *P. hyocyami*, but it remains impossible to determine whether they are *P. nicotianae*, *P. tabacina*, or a third as yet undescribed species."

Wolf² has also recently announced that *Peronospora tabacina* has been found in Brazil during the last 3 years, where it apparently is as destructive as in the United States and Australia, and behaves quite differently from *P. nicotianae*, which has been known to be present in South America since 1888. Wolf concludes that probably *P. tabacina* has only recently been introduced into South America.

Downy mildew has now been present on commercial tobacco in the United States for 10 years, and it seems possible that a study of its known behavior under the varying environmental conditions existing during the different seasons might throw some light on whether its reappearance in 1931 was coincident with the recurrence of particularly favorable weather conditions.

The history of the disease in western Florida and nearby counties of Georgia since 1931, as given in various numbers of the Plant Disease Reporter, may be briefly summarized as follows:

- 1932 Worse than in 1931, more widespread and more destructive.
- 1933 Present but less destructive than in the preceding year.
- 1934 Generally less.
- 1935 General but did little damage.
- 1936 Not important. Very few plants killed in Florida.
- 1937 Much worse; not only heavy seed bed losses in certain parts, but, for the first time, destructive losses in fields. Certain fields in Georgia lost 50 per cent and in Florida from 10 to 35 per cent.
- 1938 Not nearly so bad as in 1937. A little more than average.

¹ Wolf, F. A. Status of investigations of tobacco downy mildew. *Phytopath.* 29: 194-200. 1939. (The relevant earlier papers are cited therein.)

² Wolf, F. A. Downy mildew of tobacco in Brazil. *Phytopath.* 29: 291. 1939.

1939 Of moderate severity compared to 1937 as a severe year and 1938 as a light year.

On the basis of these records then it may be assumed that in some way 1937 was by far the most favorable year for the development of tobacco downy mildew, with 1932 probably second. There is no evidence to indicate that the pathogen has varied in aggressiveness or that the methods of tobacco culture are different. All indications point to weather conditions as determining the erratic seasonal behavior of this disease. Dixon, McLean, and Wolf³ called attention to the importance of relatively high spring and winter temperatures in relation to outbreaks of the disease and Miller⁴ to the correlation between high January temperatures and early and destructive outbreaks of tobacco downy mildew. The mean monthly temperatures, total rainfall, and number of days with more than .01 inch of rain at Quincy, Florida, for the first 4 months of each of the last 20 years, are given in table 1.

TABLE 1.—Mean monthly temperature, total precipitation, and number of days on which precipitation exceeded .01 inch, at Quincy, Florida, for the first four months of each year for the period 1920 to 1939, respectively

Temperature °F.					Total rainfall in inches				No. of days with more than .01 in. rainfall			
Year	Jan.	Feb.	Mar.	Apr.	Jan.	Feb.	Mar.	Apr.	Jan.	Feb.	Mar.	Apr.
1920	55.6	50.8	57.9	67.1	5.22	5.06	2.66	6.27	13	10	7	11
1921	56.4	56.4	66.9	65.2	3.01	2.10	2.08	2.94	8	11	8	6
1922	52.4	60.8	61.5	68.3	2.83	4.42	5.38	2.18	11	8	11	6
1923	56.6	56.3	61.6	67.2	3.88	3.75	5.05	3.88	5	10	13	12
1924	50.3	52.2	55.6	66.8	6.35	4.21	1.91	4.08	10	7	9	4
1925	55.7	56.4	61.2	67.8	8.06	2.55	2.37	1.11	17	7	6	2
1926	51.4	56.7	55.9	7.90	7.31	7.13	4.16	13	5	11	5
1927	54.1	63.8	62.3	70.5	0.24	3.71	2.04	1.18	2	8	7	5
1928	51.0	55.2	63.0	63.1	1.36	6.93	6.89	14.76	4	12	15	7
1929	56.4	57.4	64.7	70.0	5.23	2.85	4.03	4.91	13	11	8	10
1930	54.0	58.9	56.0	67.4	7.53	3.47	6.67	4.76	11	5	13	6
1931	51.2	54.4	56.6	64.9	2.67	2.57	4.68	1.15	10	9	8	4
1932	62.2	64.2	55.6	66.4	7.56	1.80	4.99	1.06	9	7	10	5
1933	57.0	55.0	60.4	65.1	4.43	6.63	5.35	12.93	9	17	5	13
1934	53.4	49.6	58.1	66.0	2.33	3.62	3.66	2.62	12	11	16	10
1935	53.1	53.8	65.2	2.23	5.64	1.96	4.50	8	8	7	10
1936	51.7	50.9	62.4	66.2	5.97	6.04	1.98	4.20	15	16	9	4
1937	64.4	54.2	57.4	64.7	3.48	5.25	5.56	6.03	12	10	9	9
1938	52.2	58.8	66.0	66.6	2.05	2.76	3.71	1.68	11	6	3	6
1939	55.0	58.9	63.0	66.6	2.71	4.49	1.34	6.33	8	12	6	8

From a comparison of the data in this table with the known history of the disease in the area, it is difficult to derive any support for the theory that the fungus was present but not destructive during the years 1922 to 1930, inclusive, and again, became important in 1931 because of more favor-

³ Dixon, L. F., R. A. McLean, and F. A. Wolf. Relationship of climatological conditions to the tobacco downy mildew. *Phytopath.* 26: 735-759. 1936.

⁴ Miller, P. R. January temperatures in relation to the distribution and severity of downy mildew of tobacco. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 21: 260-266. 1937.

able environmental conditions. On the obviously reasonable assumption that marked deviation from the conditions obtaining in the very favorable years of 1937 and 1932 might be supposed to be unfavorable to the disease, it is evident that the temperature during January and February, 1931, was less favorable than that of the same months of a number of recent years. Temperatures were much less like those of 1937 and 1932 during these apparently critical months than in either 1929 or 1930.

While it seems to be agreed that, under the conditions usually obtaining in the southeastern United States, temperature probably is more important than moisture, it may be noted that, taken as a whole, January, February, March, and April, 1931, were much drier than the corresponding months of 1937 and that each of the 4 was decidedly drier than the corresponding months of 1930. The contrast between 1931 and 1937, as regards temperature and moisture in January, February, and March, is shown in figure 1. In this graph total rainfall is used, though days with rainfall would show a similar contrast. The corresponding figures for 1929 are given to show how much more closely conditions in that year approximated those of 1937. 1930 shows a somewhat similar relation but the addition of further data would confuse the graph. We are thus faced with the fact that tobacco downy mildew became noticeably abundant in

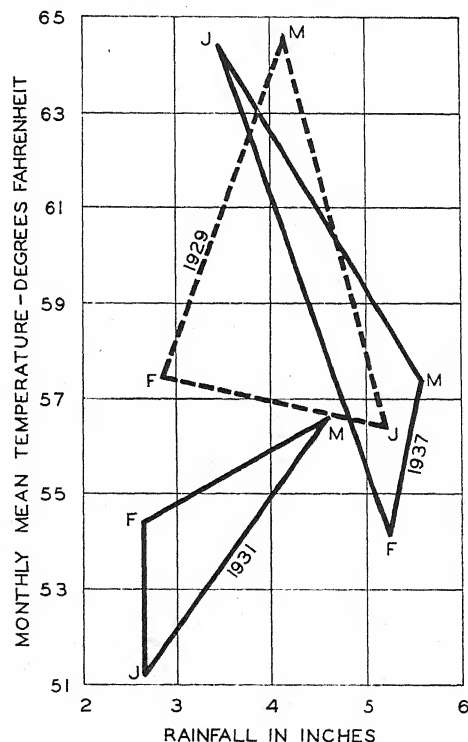


FIG. 1. Temperature and rainfall at Quincy, Florida, for January, February, and March, 1929, 1931, and 1937.

this region in a year colder and drier than its immediate predecessors, although the contrary conditions accompanied its very serious development in 1937.

The most reasonable conclusion would appear to be that the outbreak in 1931 represents a new introduction, separate and distinct from that of 1921, and that, after the outbreak of 1921, the fungus failed to become established. In other words, that the disease appeared in these two years because the fungus was introduced in those years that, as a matter of fact, were not particularly favorable to its development.

Further evidence that the spores of the fungus were widely spread in 1931 and that it may have failed to become established after it did commercial damage may be found in the sporadic outbreak in the spring of 1931 in St. James Parish, Louisiana. Prior to that date it had not, nor has it since, been observed there even though January, February, March, and April of 1932 were warmer than the corresponding months of 1931, as indicated by the weather records of nearby Donaldsonville; and the same months of 1933 also were in general, much more favorable than those of 1931.

Of course, it would be possible to argue that the disease may have persisted unnoticed during the 9 years, 1922 to 1931, in sufficient abundance to be still of commercial importance in 1931, but to anyone who has observed the disease in the field and the excitement it produces among growers, this seems about as probable as that potato blight would have gone unnoticed in Connecticut in 1844.

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SUPPLEMENTARY NOTE

Shortly after this paper was accepted for publication there appeared in a list of fungi found in Nevada during the summers of 1937 and 1938 the following record⁵: *Peronospora tabacina* Adam: on *Nicotiana attenuata* Torr. No one who knows the authors of this paper would doubt that the determination is as accurate as the available specimens permitted. One of them tells us, however, that no oospores were present in the material examined. Thus, this recent record would appear to have exactly the same status as those made in 1885, 1906 and 1914, which are cited above.

⁵ Stevenson, J. A. and W. A. Archer. A contribution to the fungus flora of Nevada. Plant Dis. Rptr. 24: 93-103. 1940.

UNSEASONABLE GERMINATION OF TELIOSPORES OF *PUCCINIA GRAMINIS TRITICI*¹

RALPH U. COTTER

(Accepted for publication April 16, 1940)

Under natural conditions teliospores of *Puccinia graminis* ordinarily require a resting period before germinating. This resting period is usually about 6 months in Minnesota for telia collected in late summer or early fall. During the past 10 years telia of all the available varieties of *Puccinia graminis* have been collected each year in Minnesota and kept outside with the expectation that germination would occur in the spring. During this 10-year period no germination was observed in any of this material before March of the year following its collection, although repeated tests were made.

With an aecial collection of *Puccinia graminis* from Loveland, Colo., sent to the writer by E. A. Lungren on September 10, 1939, was a collection of teliospores on *Elymus* and *Agropyron*. *Berberis vulgaris* plants were inoculated with these teliospores on October 2 and heavy infection resulted. In mid-November inoculations also were made with teliospores from *Agropyron smithii* Rydb., collected at Loveland subsequent to the first collection, and again heavy infection resulted. The telia in both collections gave every evidence of having been formed in the late summer of 1939, and from both the same race of *P. graminis secalis* (race 8) was identified.

Although special care had been taken to make sure that the second collection of rusted material made in Colorado would contain only current-year telia, it seemed advisable to make additional tests with carefully selected material from other sources. Accordingly, the writer collected material from the rust nurseries at University Farm, St. Paul, Minn. The wheat rust nursery was on land that had grown sugar beets the previous year; hence the possibility of contamination with straw from the previous year's crop was eliminated. The oats nursery was on land that had been so carefully prepared that there also was no possibility of collecting material from the previous year's crop. The rusted wheat and oats had been cut in early August, tied in bundles, and left outside until used for inoculating *Berberis vulgaris* in the greenhouse. Straw of durum and Marquis wheat and of Bond oats was used.

Heaviest infection resulted from inoculations made with teliospores from durum. In early November five *Berberis vulgaris* plants were inoculated. Two became heavily infected, 1 was lightly infected, and 2 did not become infected. Normal aecia developed on all infected plants and caused infection on Little Club wheat. The rust culture contained *Puccinia graminis tritici* race 44. In early December, 15 plants of *B. vulgaris*, in 3 series of 5 plants each, were inoculated at intervals of 4, 6, and 6 days, respectively,

¹ Cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station. Paper No. 1788 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

and light to heavy infection resulted on all plants in the first 2 series and on 2 of the 5 plants in the third series.

Teliospores on Marquis wheat and Bond oats apparently were not so generally viable as those on durum. Despite the fact that several sets of inoculations were made at intervals of several days during the first half of December, only 3 pycnia resulted from inoculation with teliospores from Marquis and 2 from inoculations with teliospores from oats. No aecia were produced, either as a result of "crossing" or "selfing" these pycnia.

Inoculations also were made with teliospores from *Agropyron repens* (L.) Beauv. and *Phleum pratense* L., collected in early October in Hastings, Minn., and in Wright County, Minn., respectively, but no infection was obtained.

The repeated infections obtained on barberries with teliospores from durum wheat prove conclusively that the spores of this particular collection did not require the usual 6-month resting period before germination occurred. Parallel inoculations with other collections of telia of the same and different varieties of stem rust show that the condition was not general for all collections of *Puccinia graminis tritici* nor for other varieties of rust collected from the same location. The failure of teliospores to germinate in the fall during the past 10 years is further evidence of the aberrant behavior of the teliospores on this durum wheat, which germinated 6 weeks after the initial collection was made.

The reason for the unusual behavior recorded above is not known. Johnson² says, "... it appears that either freezing, or alternate wetting and drying, has some stimulatory action on teliospore germination," and his results have been confirmed in part by Christensen.³ Such factors could hardly have been responsible for the germination of the teliospores in this durum wheat collection, however, as the fall of 1939 was one of the driest on record, with a precipitation of 1.56 inches for October, 0.02 inch for November, and 0.97 inch for December, a total precipitation of 2.55 inches for the 3 months compared with the normal precipitation of 4.33 inches. Also, the average temperature was higher than normal for this period, particularly for the months of November and December. Thus the influence of alternate wetting and drying, or freezing and thawing, could hardly have been a factor in hastening germination of the durum wheat teliospores, since very little rain fell and the temperatures were not so low as in the average year. The Colorado teliospores were evidently formed in 1939 also and they were viable despite the unusually dry weather there.

Eriksson⁴ suggested that only those teliospores produced in the late

² Johnson, T. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn. Dom. Canada Dept. Agr. Bull. 140. 1931.

³ Unpublished results by J. J. Christensen of cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station. On file at St. Paul, Minn.

⁴ Eriksson, J. Ueber die Dauer der Keimkraft in den Wintersporen gewisser Rostpilze. Centralbl. Bakt. Abt. II. 4: 376-388, 427-432. 1898.

autumn could germinate the following spring. Christensen⁵ thought that teliospores formed late in the season germinated better and sooner than those formed earlier in the year. The teliospores on the durum wheat used in the present tests appeared about 3 weeks later than those on Marquis or Hard Federation wheat at University Farm, which may possibly account for their germination at a time when those on the Marquis wheat did not germinate to an appreciable extent.

Possibly early germination of teliospores is one of the characteristics of a particular race of wheat stem rust. This is not beyond the realm of possibility, as numerous instances are known of early formation of telia by certain races of rust in the greenhouse, and it is not improbable that there may be similar racial differences with respect to the germination of teliospores.

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OBSERVATIONS ON THE VARIETAL SUSCEPTIBILITY OF APPLES TO GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

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(Accepted for publication March 2, 1940)

A severe epiphytotic of cedar-apple rust near Ithaca, New York, during the season of 1939, gave the writers an exceptional opportunity to observe the relative susceptibility of a large number of apple varieties. The little village of Forest Home, where the junior writer resides, has an unusual number of apple varieties growing within its borders. The area surveyed is in the heart of the village, covering a circle roughly $\frac{1}{4}$ mile in diameter.

In the approximate center of this area stands a red cedar 10 feet tall, which seems to have been the source of inoculum. This tree harbors 3 species of *Gymnosporangium*: *G. juniperi-virginianae*, *G. globosum*, and *G. clavipes*. No data are available as to the relative abundance of *Gymnosporangium* galls on this tree in the spring, though evidences of all 3 species were still to be found in September when the observations on the apple trees were made.

Over 40 individual apple trees were critically examined. In most cases a single tree of a variety and in a few cases 2 to 4 trees of a given variety were to be found in the area. The varietal names of several other apple trees showing infection could not be determined with certainty, and others apparently were unnamed seedlings. Out of all the trees examined there were 25 varieties, the identity of each of which could be definitely determined. The varieties York, Rome, and Jonathon were not present in the area. Specimens of diseased leaves of each affected variety were critically examined under the microscope to determine the identity of the pathogen involved in each case. Specimens from each of 3 varieties showing marked differences in lesional characters were submitted to F. D. Kern and to Paul R. Miller, both of whom pronounced our pathogen to be *Gymno-*

⁵ See footnote 3.

sporangium juniperi-virginianae. All specimens from the other varieties agree in their microscopic characters with those submitted to the experts. It, therefore, appears that *G. juniperi-virginianae* is the only species involved in this rust attack on the apple trees in the area under discussion.

Trees of 20 of the varieties here reported are on the junior writer's own property. He sorted the fruit from most of these trees but found only 1 apple, a Sutton, showing a rust lesion, and this, apparently, of *Gymnosporangium juniperi-virginianae*. The absence of rust lesions on the

TABLE 1.—Comparison with records of other observers on the susceptibility of apple varieties to *Gymnosporangium juniperi-virginianae*

Variety	Observers ^a						
	1	2	3	4	5	6	7
Baldwin	I ^b	c		I	VR		
Chenango Strawberry	I		I		VR		
Dutchess of Oldenburg	I				R		
Hubbardston	I				R		
Lady	I						
McIntosh	I				R		
Northern Spy	I	S			R		
Pound Sweet	I				R		
Red Astrachan	I		I		R		R
Red Delicious	I				VR		
Rhode Island Greening	I		R		R		
Sutton	I						
Wagener	I		I		R		
Yellow Transparent	I		I	I	R	R	I
Golden Delicious	VR				R	S	
Mother	VR						
Roxbury Russett	VR				R		
Westfield Seek-No-Further	R				R		
Ensee	S						
Tompkins King	S				S		
Summer Rambo	VS				R ^d		
Twenty Ounce	VS		S		VS		
Wealthy	VS			VS	VS		VS
Winter Banana	VS				S		
Yellow Bellflower	VS				VS		

^a 1. Whetzel and Niederhauser. Ithaca, N. Y., October, 1939.

2. Reed and Crabill. Virginia, 1915. (Virginia Agr. Exp. Stat. Tech. Bull. 9: 39).

3. Chester. Delaware, 1896. (Del. Agr. Exp. Stat. Ann. Rept. 8: 63-69).

4. Giddings and Berg. West Virginia, 1915. (W. Va. Agr. Exp. Stat. Bull. 154: 70).

5. Bliss. Iowa, 1933. (Iowa State Coll. Agr. Exp. Stat. Res. Bull. 166: 368-370).

6. Miller, P. R. Tennessee, 1934. (U. S. Dept. Agr. Pl. Dis. Rprtr. 18: 161).

7. Stewart and Carver. Iowa, 1896. (New York (Geneva) Agr. Exp. Stat. Rept. 14: 534-544).

^b Key: I—immune, no lesions observed.

VR—very resistant; flecks only.

R—resistant; pycnia, no aecia.

S—susceptible; few aecia.

VS—very susceptible; aecia numerous.

^c Note:—The varieties we report as immune might well show flecks or even pycnia with very favorable conditons for infection under which Bliss tested these same varieties.

^d Bliss apparently had the Rambo, a red apple.

fruits is remarkable but is probably to be attributed to the stage of fruit development at the time inoculation occurred.

Although galls of *Gymnosporangium globosum* and branch swellings of *G. clavipes* were abundant on the cedar tree, not a single lesion of either was observed in our rather extensive and careful scrutiny of the apple trees examined. What peculiar combination of weather conditions and stage of development of the apple foliage and fruits may have been responsible for the absence of lesions of these 2 species, it is impossible to suggest.

VARIETAL REACTIONS OF CERTAIN APPLE VARIETIES TO *GYMNOSPORANGIUM*
JUNIPERI-VIRGINIANAE AT FOREST HOME, ITHACA, N. Y., 1939

Immune. Yellow transparent, Wagener, Lady, McIntosh, Dutchess of Oldenburg, Rhode Island Greening, Chenango Strawberry, Baldwin, Northern Spy, Red Astrachan, Sutton (Beauty), Red Delicious, Hubbardston, Pound Sweet (?).

Very resistant. (Flecks only, few)—Golden Delicious, Mother, Roxbury Russett.

Resistant. (Pycnia, no aecia)—Westfield Seek-No-Further.

Susceptible. (Aecia relatively few)—Ensee, Tompkins King.

Very Susceptible. (Aecia abundant and well developed)—Twenty Ounce, Yellow Bellflower, Winter Banana, Wealthy, Summer Rambo.

That more than one pathogenic race of *Gymnosporangium juniperi-virginianae* is here involved seems doubtful. A comparison of the reactions of the varieties herein recorded with records made by other observers suggests that this race may be the same as that reported by Bliss in Iowa for the years 1928–1930 (See Bliss 1933: 339).

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PHYTOPATHOLOGICAL NOTES

*An Unusual Telial Collection of Puccinia graminis.*¹—One of the unusual things encountered in the stem-rust hybridization has been the behavior of occasional aberrant telial collections. Such a collection, No. 7990 of the rust collections, was sent in from Winona County, Minnesota, February 28, 1934. Although heads were missing, the sample had been labelled stem rust on *Agrostis* by the collector. Barberry was inoculated with the teliospores from this collection, and barley but not red-top became heavily infected when inoculated with the aeciospores produced. The rust died before being identified. Selfed aecia failed to infect either barley or *Agrostis*, no other hosts being inoculated.

Two years after collection this same material was again used to inoculate barberry, and barley was inoculated with the resultant aeciospores. This

¹ Cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station. Published with the approval of the Director as Paper No. 1800 of the Minnesota Agricultural Experiment Station.

uredial culture proved to be race 11 of *Puccinia graminis tritici*, a rather surprising result, because we do not expect to obtain the *tritici* variety from a collection that is not on a wheat host. Crosses between this *Agrostis* collection and other rusts, however, produced inexplicable results. As shown in table 1, a cross between the *Agrostis* rust collection and a wheat rust col-

TABLE 1.—*Progeny from teliospore collection No. 7990 of Puccinia graminis on Agrostis and from crosses with other varieties of Puccinia graminis*

<i>Agrostis</i> (7990)	natural aecia	—Barley susceptible, not identified
"	" selfed aecia	—No infection on barley or <i>Agrostis</i> ; no other hosts inoculated
"	" natural aecia	— <i>P. graminis tritici</i> 11
"	" " "	— <i>P. graminis secalis</i> , race unknown
"	" × wheat (7963)	— <i>P. graminis tritici</i> 103 and 160
"	" " (6823)	—Barley susceptible, not identified; no infection on wheat
"	" × <i>Agrostis</i> (7957)	— <i>P. graminis tritici</i> 39
"	" × " "	— <i>P. graminis tritici</i> 39; <i>P. graminis secalis</i> 11
"	" × <i>Elymus</i> (8724)	— <i>P. graminis tritici</i> 36 and 161; <i>P. graminis secalis</i> 7
Reciprocal Crosses		
<i>Elymus</i> (8724)	× <i>Agrostis</i> (7990)	— <i>P. graminis tritici</i> 17 and 36; <i>P. graminis secalis</i> 8
"	" × " "	—Barley weakly infected (<i>agrostidis</i> ?)

lection (7963) resulted in races 103 and 160 of *P. graminis tritici*; a second cross with a different wheat rust collection produced a rust that infected barley but not wheat. This rust died before being identified: Possibly it was a rust that could infect barley only, but it could also have been a *secalis* race. Crosses between this *Agrostis* rust collection and another collection on *Agrostis* resulted in race 39 of *P. graminis tritici* and race 11 of *P. graminis secalis*. A cross between the *Agrostis* rust collection and a *secalis* race on *Elymus* produced two races of *P. graminis tritici* and one of *P. graminis secalis*. From a reciprocal cross, a *secalis* race on *Elymus* with the *Agrostis* collection, two races of *tritici*, and one of *secalis* came out; from a second reciprocal cross a rust was produced which did not infect barley heavily. This last might possibly have been an *agrostidis* race, since both the *tritici* and *secalis* varieties of stem rust can attack barley heavily.

The *tritici* races 103 and 160 differed considerably from races 15 and 52 that were identified from parent wheat collection No. 7963. Races 103 and 160 are less virulent than either of the races in the paternal parent. They attack Little Club wheat weakly and indeterminately (X reaction), and Einkorn weakly and heavily, respectively. Another characteristic of these races is the early and abundant formation of telia on the inoculated seedlings, the telia forming within 3 weeks after the initial appearance of uredia.

whereas, under normal greenhouse conditions, telia do not usually appear in other rust cultures before 6 weeks or more have elapsed. It may be of interest to know that race 160 was first identified from a cross between *Puccinia graminis secalis* race 11 and an *Agrostis* rust collection from Wisconsin.

In the cross made between the *Agrostis* rust and the *Elymus* rust a new race of *Puccinia graminis tritici* appeared. This race, No. 161, differs from race 21, which it most nearly resembles, by the reaction of Little Club or Jenkin wheat. To race 21, Little Club is very susceptible; to race 161, Little Club is intermediate in reaction, at times ranging from immunity to susceptibility in the reactions in the same pot of seedlings. The differences were consistent when the two forms were growing on Little Club wheat in adjacent booths in the greenhouse. Another difference is that race 161 attacks Hope wheat more heavily than the typical race 21.

Summarizing, this collection of telia on grass stems said to be those of *Agrostis* has produced a *tritici* strain from naturally formed aecia, and from crosses with *tritici*, *secalis*, and *agrostidis* varieties of *Puccinia graminis* has produced other *tritici* and *secalis* rusts and possibly an *agrostidis* rust. One of the *tritici* races obtained was different from anything previously identified. While the identity of the rust on this grass collection was not established beyond a doubt, it was either the *agrostidis* rust or a hybrid so different from any other *tritici* or *secalis* rust previously encountered as to be unable to attack barley, which has proved susceptible to all races of the *tritici* and *secalis* varieties tried. These results make the Winona collection a most unusual one from two standpoints: First, that a *tritici* race was obtained from what was not a wheat collection; and, second, that a new *tritici* race was obtained from a cross between this collection and a *secalis* collection.—RALPH U. COTTER, University Farm, St. Paul, Minnesota.

*A Simple Single-spore Isolator.*¹—To make single-spore isolations with a glass needle, an attachment was devised to be clamped to the holder of the substage condenser. While the use of the substage mechanism for vertical motion in single-spore isolation is not new,^{2,3} the device is so satisfactory in operation and of such economical construction (about \$12.00), in comparison with others on the market, a description of it may be of interest.

The general techniques for single-cell isolations with a needle are adequately reviewed,^{2,4} and only the modifications necessary for the operation

¹ A contribution from the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Northeastern States and the Department of Botany, the Pennsylvania State College, State College, Pa. Thanks are due to Dr. H. L. Yeagley, of the Physics Department of the Pennsylvania State College, for his kindness in constructing the device.

² Dickinson, S. The technique of isolation in microbiology. *Phytopath.* 23: 357-367. 1933.

³ Edgerton, C. W. A method of picking up single spores. *Phytopath.* 4: 115-117. 1914.

⁴ Hildebrand, E. M. Techniques for the isolation of single microorganisms. *Bot. Rev.* 4: 627-664. 1938.

of the attachment need be described. Figure 1 gives the details of construction, and figure 2 shows the device in position on the microscope. It may

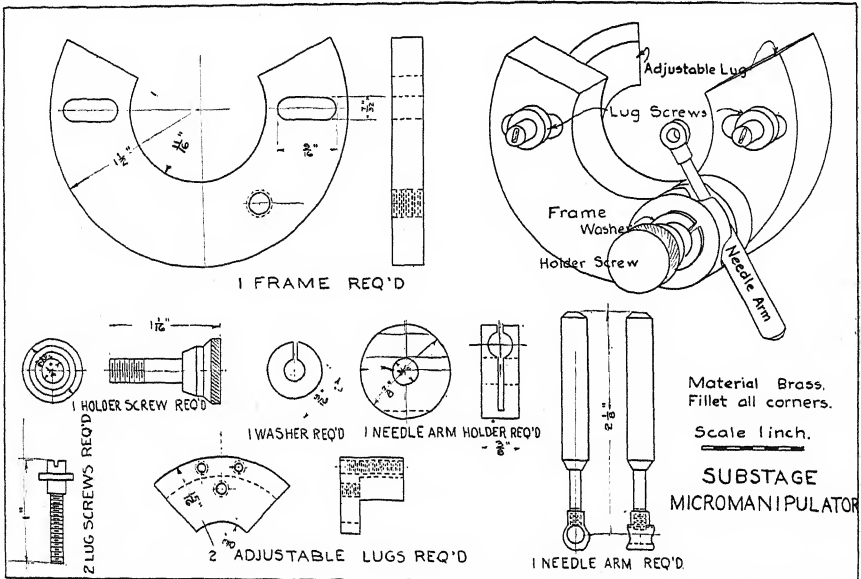


FIG. 1. Diagram to scale of parts required for attachment to substage condenser for single-spore isolation.

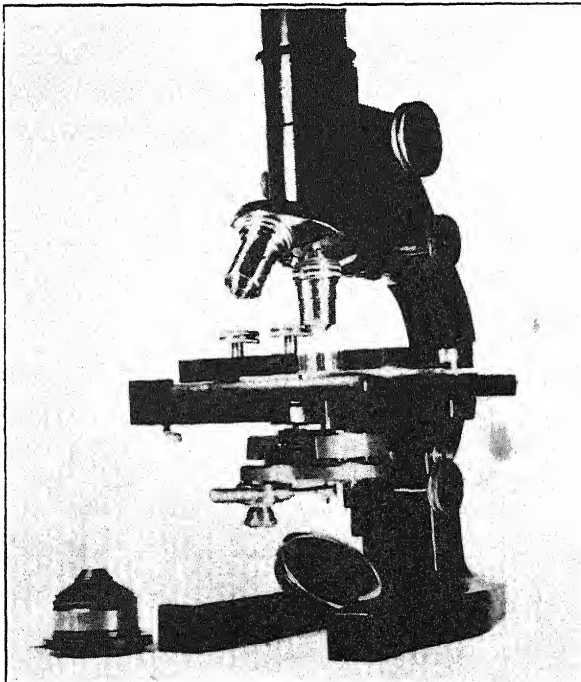


FIG. 2. Single-spore isolator, glass needle, isolation chamber, and mechanical stage in position on microscope.

be seen that the condenser is removed and the attachment is clamped to the holder of the condenser. The glass needle fits into the end of the projecting arm and the point extends through the aperture of the stage. The isolation chamber consists of a van Tieghem cell, glued to the center of a piece of wood the size of an ordinary microscope slide. A hole, the same diameter as the inside of the cell, is made to permit the passage of light and the needle. The point of the needle is centered in the microscope field by horizontally and radially manipulating the arm holding it. The spring washer shown in figure 1 applies sufficient tension to permit easy adjustment of the arm and to hold it in position when adjusted. The spores, which have been placed on a film of agar on a cover slip inverted on the van Tieghem cell, are brought into position over the needle with the mechanical stage.

The attachment is easily fitted to various styles of microscope. Satisfactory isolation has been made of single spores as small as 7μ in length. Plenty of light is available without the condenser, if a lamp be used.—S. J. P. CHILTON, U. S. Regional Pasture Research Laboratory, State College, Pa., and C. C. WERNHAM, Department of Botany, The Pennsylvania State College, State College, Pa.

*Infectivity of Tobacco Mosaic Virus in Liquids over 14 Years Old.*¹—The virus of tobacco mosaic can retain its infectivity in air-dried leaves for more than 52 years.^{2, 3} Published reports on the retention of infectivity of the virus in liquids usually record the time in weeks or months probably because older material is unavailable for study. Dixon,⁴ however, found that tobacco-mosaic extract, covered with a layer of toluene, in a tightly stoppered flask, was infectious after $5\frac{1}{4}$ years.

In 1925, mosaic extracts were prepared and stored as follows in stoppered⁵ Erlenmeyer flasks in a closed cabinet at room temperature:

Flask 1. Frozen mosaic-tobacco suckers from the field, ground to a pulp and mixed with water. Stored October 25, 1925.

Flask 2. Air-dried mosaic tobacco leaves crushed and water added. Stored October 25, 1925.

Flask 3. 200 grams of mosaic-affected green leaves ground to a pulp and 500 cc. water added. 200 cc. of this suspension stored on December 17, 1925, after addition of 5 cc. of benzene.

Flask 4. Like flask 3, 5 cc. of xylene added.

Flask 5. Like flask 3, 5 cc. of toluene added.

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by the permission of the Director.

² Johnson, E. M., and W. D. Valleau. Mosaic from tobacco one to 52 years old. Ky. Agr. Exp. Stat. Bull. 361: 264-271. 1935.

³ Thornberry, H. H., W. D. Valleau, and E. M. Johnson. Inactivation of tobacco mosaic virus in cured tobacco leaves by dry heat. Phytopath. 28: 129-134. 1938.

⁴ Dixon, B. F. Tobacco and tomato mosaic. Sci. 62: 398. 1925.

⁵ Cork stoppers were used. When the flasks were opened the volume of the liquid was found to have decreased about a third and, of the preservatives used, the odor of xylene only was detectable.

In January and February 1940, half leaves of a white Burley tobacco hybrid containing the necrotic factor from *Nicotiana glutinosa*, were inoculated, by the glass spatula method, with liquid from each of the 5 flasks and with a fresh extract of tobacco-mosaic virus of the dilution originally used in flasks 3, 4, and 5. Two days later necrotic spots appeared on all leaves rubbed with virus extracts containing benzene and xylene and from fresh extract. The number of necrotic spots developing from the fresh virus extract was 2.5 to 3 times greater than the number developing from the extracts containing benzene and xylene. A few necrotic spots developed on leaves rubbed with extracts containing no preservative and from that containing toluene, indicating that the virus was still infectious but the concentration low (Table 1). To be certain that necrotic spotting was not chemical injury, isolated necrotic spots, removed with a cork borer from each inoculated plant, were crushed in M/10 di-sodium phosphate and rubbed on leaves of white Burley tobacco with a glass spatula. Tobacco mosaic mottling developed in 5 to 7 days in all the inoculated plants, indicating that the necrotic spots were the result of tobacco-mosaic virus that had retained its infectivity in liquid extracts for over 14 years (Table 1).

TABLE 1.—Retention of infectivity of tobacco-mosaic virus in liquid extracts, 14 years old

Flask number	Necrotic spotting Burley half leaves inoculated	Necrotic spots	Necrotic spots per half leaf	White Burley tobacco inoculated from single spots	
				Number	Number developing mosaic
	<i>Number</i>	<i>Number</i>	<i>Average</i>		
1	18 ^a	5	0.28	4	4
2	18 ^a	4	0.22	3	3
3	9 ^b	584	65.0	10	10
4	9 ^b	726	80.0	10	10
5	6 ^b	6	1.0	6	6
Fresh mosaic extract	3	606	202.0

^a 4 tests on different days.

^b 2 tests on different days.

Often it is desirable to keep tobacco mosaic virus for further studies. Usually the virus is preserved in dried leaves or in frozen extracts. Dried leaves often mold or are eaten by insects. Cold storage facilities are not available in most laboratories. The use of benzene or xylene as preservatives of tobacco-mosaic-virus extracts overcomes these disadvantages and appears to be a reasonably safe method for preserving the virus for several years.—E. M. JOHNSON and W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

*Variation in the Tolerance of Certain Physiologic Races of Actinomyces scabies to Hydrogen-ion Concentration.*¹—The development of common scab of potatoes is usually favored by an alkaline or slightly acid soil. It is commonly accepted that scab will not develop to any extent on potatoes growing in soils with a pH of less than 5.0. Considerable damage does occur, however, in soils with a pH of 5.4.

In 1937, *Actinomyces scabies* was isolated from potato tubers growing in soils with pH 5.4 and 6.8, respectively. The isolates were designated as Nos. 23 and 66. The latter was isolated from tubers growing in the more acid soil and could not be distinguished from No. 23 when single-spored and grown on potato-dextrose agar adjusted to pH 7.0. Both isolates were grown on potato-dextrose agar adjusted to different pH values. Previous experiments had shown that potato-dextrose agar containing 0.5 per cent

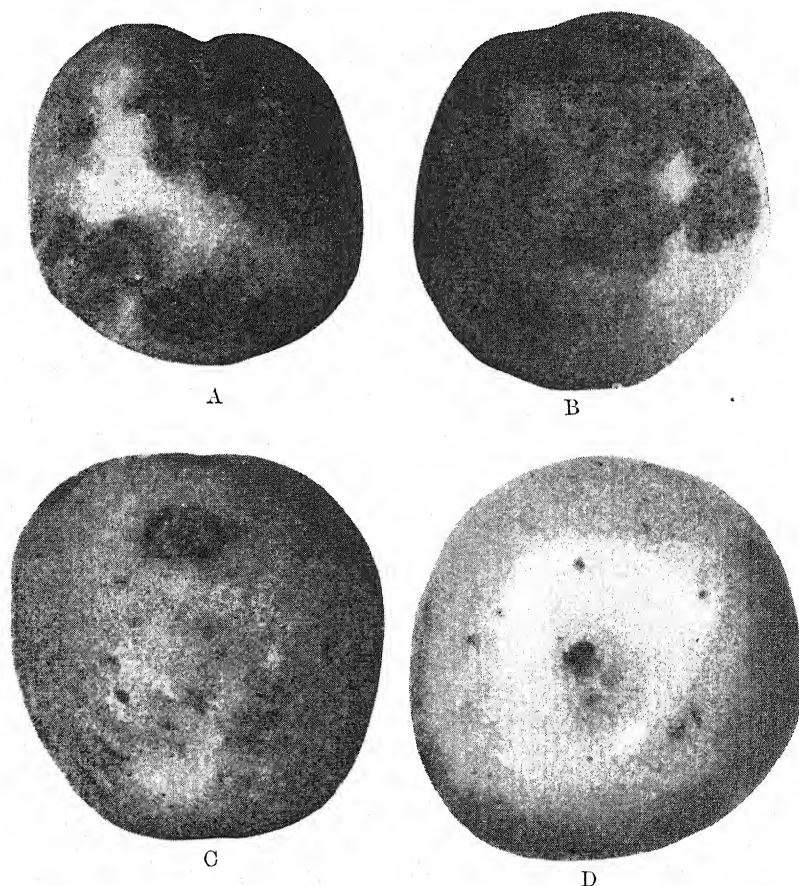


FIG. 1. Scab infection produced on tubers of Green Mountain and Katahdin potato varieties by isolates Nos. 23 and 66 of *Actinomyces scabies*. A. Green Mountain inoculated with isolate No. 23. B. Katahdin inoculated with isolate No. 23. C. Green Mountain inoculated with isolate No. 66. D. Katahdin inoculated with isolate No. 66.

¹ Published with the approval of the Director as Paper No. — of the Minnesota Agricultural Experiment Station.

dextrose supported growth of these isolates when adjusted to pH 7.0. When isolates Nos. 23 and 66 were placed in this medium adjusted to pH 4.5, neither of them grew. Isolate No. 66 grew readily at pH 5.0 and also at 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5. Isolate No. 23 did not grow at pH 5.0 and grew only slightly at 5.5, but at pH 6.0 to 8.5 it grew at approximately the same rate as isolate No. 66. When these two isolates were compared with seven from various sections of the United States and one from Australia on the same medium at the same pH values, only isolate No. 66 grew readily at pH 5.0.

Both isolates were pathogenic, as shown by results of inoculating tubers growing in the greenhouse (Fig. 1). Isolate No. 66 produced a shallow, blister-like pustule on Green Mountain and Katahdin varieties, whereas isolate No. 23 produced a deep, severe-type pustule on the same varieties grown in a soil with a pH reaction of 6.8. The degree of pathogenicity of isolate No. 66 in soil with a pH of 6.8 was practically the same as that in the soil from which it was isolated, the soil with a pH of 5.4. Thus there is little probability that soil reaction was a factor determining pustule type. In general, when the pH reaction is below 5.4 in the field, very little deep scab is found, the shallow or russet type usually being the only one.

These studies of the pathogenicity of isolates Nos. 23 and 66 indicate that they are physiologic races of *Actinomyces scabies*. They also can be differentiated on the basis of pH tolerance in culture and probably in the field, as well as by their differences in pathogenicity.—LAWRENCE A. SCHAAAL.²

Phytophthora cactorum as a Cause of Root Rot in Sweetclover.¹—A destructive root rot of sweetclover has been found in Alberta, which was considered similar if not identical with the *Phytophthora* root rot of the same host reported by Jones² in the United States. A highly pathogenic species of *Phytophthora* isolated from the diseased roots was identified by S. F. Ashby, Director of the Imperial Mycological Institute, England, as a strain of *Phytophthora cactorum* (Leb. and Cohn) Schroet. Jones has since tested an isolate sent to him from Alberta and compared it with his cultures. As a result, he agreed that the fungus in the United States is *P. cactorum*, and not *P. megasperma*, as previously reported. The latter species, while occurring on sweetclover, is nonpathogenic or, at most, weakly pathogenic to this host. Sweetclover, therefore, is apparently a new host for *P. cactorum*.

In Alberta, *Phytophthora* root rot of sweetclover has been found on both irrigated and nonirrigated land in several widely separated districts. Most of the damage observed so far has been confined to scattered plants in fields

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¹ Contribution No. 619, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa.

² Jones, Fred Reuel. Evidence of resistance in sweetclover to a *Phytophthora* root rot. *Phytopath.* 29: 909-910. 1939.

and along the roadside. However, *Phytophthora* root rot may become important in Alberta, since in a field near Lethbridge last June about 20 per cent of the plants were dead or dying from this cause. The host appears to be highly susceptible both in early spring and during the growing season, with symptoms similar to those described by Jones.³

The pathogenicity of several isolates of *Phytophthora cactorum* from sweetclover has been tested in the field during the summer. In these experiments roots of sweetclover and alfalfa were inoculated by inserting small portions of mycelial inoculum beneath the bark. All the isolates proved highly virulent on sweetclover but did not attack alfalfa. The pathogen invaded the sweetclover roots very rapidly, causing many of the plants to wilt and die within 10 days after inoculation. Preliminary experiments indicated that mature plants were more susceptible than seedlings, since plants inoculated at the flowering stage in their second year of growth had an average infection rating of 90 per cent, as compared with 60 per cent for 2-month-old seedlings. Further studies are now in progress and will be reported later.—M. W. CORMACK, Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton.

Isolation of Ceratostomella ulmi from Scolytus multistriatus Adults Stored at Different Temperatures.—In laboratory and field studies relating to the Dutch elm disease fungus, *Ceratostomella ulmi* (Schwarz) Buisman, it is often desirable to determine whether or not bark beetles and other insects are contaminated with this fungus. Frequently, field-collected specimens are so numerous that they cannot be cultured immediately upon receipt. Therefore, this experiment was conducted to ascertain what temperatures were most satisfactory for storage.

Scolytus multistriatus (Marsh.) adults were contaminated by contact with Petri-plate cultures of *Ceratostomella ulmi* and placed in new individual gelatin capsules. These capsules were mixed and divided into 22 lots of 50 each. Two lots were cultured immediately by a modification of the method described by Walter¹ as a check on the contamination of the beetles. Four lots were stored at each of the following temperatures: 70°, 60°, 40°, 28°, and -10° F. One lot from each storage condition was removed and cultured at the end of 30, 60, 90, and 120 days, respectively.

Ceratostomella ulmi was recovered from 100 per cent of the beetles cultured immediately as a check. All but 2 beetles of the 10 lots cultured 30 and 60 days after storage yielded the fungus. At the end of 90 days *C. ulmi* was recovered from only 60 per cent of the beetles stored at 70° F., and 58 per cent of those stored at 60° F.; at the end of 120 days the recoveries were zero and 22 per cent, respectively, for these temperatures. The fungus was recovered from all the beetles stored at the lower temperatures for 90 and 120 days.

³ See footnote 2.

¹ Walter, J. M. Technique advantageous for the isolation of *Ceratostomella ulmi* from bark beetles. (Abstract) *Phytopath.* 25 (1): 37-38. 1935.

Three additional lots of beetles, stored at -10° F., were cultured at the end of 1, $1\frac{1}{2}$, and $2\frac{1}{2}$ years. Recoveries of *Ceratostomella ulmi* were 100, 98, and 100 per cent (the latter based on 44 beetles), respectively.

Any of the storage conditions studied were adequate if the material was not kept beyond 60 days. In all cases, however, cultures of beetles stored at -10° F. were most satisfactory on the basis of rapidity of coremial development and freedom from contaminants.—C. S. MOSES, Division of Forest Pathology, Bureau of Plant Industry, and CLARENCE H. HOFFMANN, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

REPORT OF THE 1940 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1940 Annual Meeting of the Southern Division of The American Phytopathological Society was held in connection with the Annual Meeting of the Association of Southern Agricultural Workers on February 7-9, inclusive, in Birmingham, Alabama. A total of 46 papers were presented at the four general sessions, which made the fullest program the Southern Division has ever presented. In addition to these four sessions, a joint session was held with the Southern Section of the American Society for Horticultural Science, in which consideration was given to some leading pathological problems on vegetable crops. Also, the last afternoon of the meeting was devoted to holding the third National Nematode Conference. Approximately 45 plant pathologists were in attendance at the meeting.

A short business session was held on the morning of February 9, when the following officers were elected:

President, C. H. Arndt
Vice-President, A. L. Harrison
Counselor, G. M. Armstrong

Abstracts of papers presented at the meeting follow.

LUTHER SHAW
Secretary-Treasurer

A Photo-electric Method and Its Use for Determination of Fungus Growth Rates. L. A. ADAIR and ELIZABETH J. MOORE. A photo-electric apparatus is described for determining the difference in density and size between fungus colonies. Such readings are shown to give good agreement with determinations based on dry weights, using the cotton root-rot organism, *Phymatotrichum omnivorum*. Readings can be made by this method at the rate of 150 per hour.

Infection of Cotton Seedlings in the Greenhouse by Phymatotrichum omnivorum. LESTER M. BLANK. Frequent occurrence of healthy cotton seedlings or young plants in areas of severe root-rot infestation suggested possible seedling resistance to *P. omnivorum*. To obtain information on susceptibility of seedlings, experiments were conducted in the greenhouse. Cotton seedlings, in Houston clay, were exposed to infection from sclerotia of *P. omnivorum* introduced into the soil near roots of seedlings. Such inoculations were made 18, 25, and 33 days after planting of the seed. Soil temperature was constant in all at $26-28^{\circ}$ C., whereas soil moisture was varied at three levels, following inoculation. With the two highest soil moistures (approx. 36 and 45 per cent of the maximum water-holding capacity), above ground symptoms of disease were evident in certain seedlings 10 days after inoculation, regardless of age of seedling at time of inoculation. The lowest soil moisture (27 per cent) was unfavorable for seedling growth; infection and collapse of the seedlings occurred infrequently, only 3 per cent disease being observed in the low moisture group after 24 days' exposure to infection.

Statistical analysis of results showed two highest soil-moisture groups did not differ significantly in amount of disease, but both differed significantly from the lowest moisture. Disease was significantly more abundant in the oldest group of plants (33 days at time of inoculation) than in either of the younger groups. Its rate of development was faster in older plants than in younger ones.

In a subsequent experiment, with soil moisture at 27 and 33 per cent, inoculated 10 days after planting, approx. 35 per cent of seedlings had collapsed within 18 days after introduction of sclerotia into the soil. Similar infection experiments conducted with seedlings grown in sand cultures were conducted successfully although the percentage of diseased plants is somewhat lower than that obtained on Houston clay.

Growth Response of Phymatotrichum omnivorum to Certain Inorganic Nitrogens. LESTER M. BLANK and PAUL J. TALLEY. Mean weights of fungal mats grown on standard nutrient solution with equivalent amounts of nitrogen derived from NH_4NO_3 , KNO_3 , $\text{Mg}(\text{NO}_3)_2$, $\text{KNO}_3 + \text{Mg}(\text{NO}_3)_2$, or $\text{Ca}(\text{NO}_3)_2$ are greater than those obtained with nitrogen from $(\text{NH}_4)_2\text{HPO}_4$, NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2 + \text{NH}_4\text{Cl}$, $(\text{NH}_4)_2\text{HPO}_4 + (\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{HPO}_4 + \text{NH}_4\text{Cl} + (\text{NH}_4)_2\text{SO}_4$, or $\text{NH}_4\text{Cl} + (\text{NH}_4)_2\text{SO}_4$. Of the ammoniacal sources of nitrogen $(\text{NH}_4)_2\text{HPO}_4$, or combinations in which it is present, consistently yield heavier mats than do NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, or combinations of the two. Determinations of pH values of solutions at time of inoculation and at time of harvest suggest that rapid shift from approximate neutrality to a strongly acidic condition is responsible for poor growth with ammoniacal nitrogen sources. The addition of CaCO_3 or Na_2CO_3 to solutions with above nitrogen sources has little or no beneficial effect on amount of growth in solutions with nitrate nitrogen, but generally increases growth in solutions with ammoniacal nitrogen. MgCO_3 exhibits depressing effect on growth with NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, or MgNO_3 , increases amount of growth with other NH_4 sources, and usually has little effect on amount of growth with other NO_3 sources. With nitrogen derived from NH_4NO_3 , $(\text{NH}_4)_2\text{HPO}_4$, or $(\text{NH}_4)_2\text{SO}_4$ it has been demonstrated that nitrogen concentration used in these experiments is well below upper limits of growth tolerance. Over a range of concentrations of nitrogen (equivalent to .00625 to .0750 M. NH_4NO_3), CaCO_3 at .0125 M. is effective in increasing fungal growth with $(\text{NH}_4)_2\text{HPO}_4$, and $(\text{NH}_4)_2\text{SO}_4$ but had little or no effect on solutions containing NH_4NO_3 . Results indicate main difference in the utilization of ammoniacal and nitrate nitrogen under these conditions is closely associated with resulting shifts in pH values of nutrient solutions. To obtain additional information on this point experiments employing buffered solutions were conducted. In experiments with nitrogen derived from NH_4NO_3 , NH_4NO_2 , and KNO_3 the yields are in the approximate ratio of 10:5:3, respectively.

Control of Sclerotiniase of Celery on Florida Muck. A. N. BROOKS. Pink rot of celery (*Sclerotinia sclerotiorum*) has, during periods of cool moist weather, caused much damage to the crop in the muckland area near Sarasota. Controlled-temperature experiments showed that for mycelial growth the optimum is 75° G., the minimum below 45° and the maximum between 83° and 89°. Above 70° F. sclerotia produced no apothecia, below 70° sclerotia produced apothecia within 28 to 34 days. Thus, initial development of apothecia in the field has been predicted to occur 28 to 34 days after the mean daily temperatures have dropped below 70° F. Sclerotia buried deeper than 3 in. in the soil produced only stipes, unable to reach the surface and mature apothecia. Deep plowing gave poor control because all sclerotia were not buried to the required depth. Flooding the muck for 6 to 8 weeks in the summer destroyed over 90 per cent of the sclerotia in the soil. Of various chemicals applied to the soil, only calcium cyanamid destroyed sclerotia. Post-harvest or pre-planting applications were equally effective. Of various sprays and dusts applied to the celery plants and immediately adjacent soil, none gave complete control, although there were variations in the effectiveness of the different materials in lessening incidence of the disease.

Field Results With Gravity-graded Cotton Seed. K. STARR CHESTER. Cotton seeds were acid-delinted and divided into the fractions that sank ("heavy") and floated ("light") in water, respectively. These fractions, together with aliquots of nondelinted, "fuzzy" seed and nongraded ("whole") delinted seed, were planted on each of 2 dates in 1938 and 1939. Each planting consisted of 4 50-foot rows of each of the 4 seed types, planted at 5 seeds per hill. In 1939 the 2 plantings also were repeated at the rate of 2 seeds per hill. The percentages of successful emergence and yields of seed cotton were recorded.

Averaging all experiments, the "heavy" seed showed 34, 52, and 159 per cent better emergence than the "whole," "fuzzy," and "light" seed respectively. These improved stands were reflected in yield increases of 8, 41, and 58 per cent respectively. Under adverse weather conditions which produced stand failures, the "heavy" seed alone gave useable stands. "Heavy" seed, planted at 2 per hill, produced stands that required no chopping, and outyielded chopped stands of the other types of seed planted at 5 seed per hill.

The advantages of delinting and grading were considerably greater than those of dusting fuzzy seed in the cooperative seed treatment tests ("A") in adjacent rows.

Resumé of Five Years Spraying with Low-lime Bordeaux Mixture and Zinc Sulphate to Control Pecan Scab and Rosette Diseases. JOHN R. COLE. Pecan scab is one of the most important limiting factors in nut production, especially in the Southeastern States, where its control is of prime importance to the pecan industry. The fungus causing scab, (*Cladosporium effusum* (Wint.) Demaree) is strongly parasitic, invades only young or growing tissues, and infections result in elongated or circular brown or black lesions one-eighth to one-quarter inch in diameter. To control scab, begin spray operations between April 10-20 in South Georgia, using 4-1-100 Bordeaux mixture for the first or prepollination application, followed by 3 applications of 6-2-100 Bordeaux mixture. These applications are made at intervals of 3 to 4 weeks with the last one coming about the middle of July. By following this spray program from 1935 to 1939, inclusive, the sprayed trees have averaged 55 lb. of good quality nuts, while the nonsprayed checks have averaged only 10 lb. of low grade nuts.

Where scab-susceptible varieties also are rosetting add 2 pounds of zinc sulphate (analyzing approximately 36 per cent zinc) to the last 3 applications of Bordeaux mixture. Rosette was completely overcome by spraying for two years with this solution. The disease was also prevented by adding zinc sulphate to either of the last 3 applications annually.

Effect of Girdling and Topping of Cotton Plants on Survival of Phymatotrichum omnivorum on the Roots. WALTER N. EZEKIEL. In work reported previously, girdling cotton stems during the period of rapid growth reduced survival of *P. omnivorum*, coincident with decrease of alcohol-soluble solids in the roots. Further tests of girdling and topping were set up June 30, 1939, in 18 small plots at the advancing edges of root-rot spots in a cotton field of Houston black clay soil. After 18 or 25 days, the fungus apparently was killed on all plants from 3 of the 6 plots with plants whose tops had been removed, but did not disappear from plots with untreated plants or girdled plants. *P. omnivorum* was recovered, in this experiment, from 31.4 per cent of check plants, from 21.8 per cent of girdled plants, and from 9.5 per cent of topped plants. Including also the results of tests in 1938, percentages of plants from which the fungus was recovered were: check plants, 31.8; girdled plants, 21.3; and topped plants, 14.5. The roots were apparently changed by both treatments sufficiently to reduce not only the percentage of plants on which *Phymatotrichum* survived but also the extent and profusion of growth of the fungus from those roots on which it was still alive.

Relation of Age of Cotton Plants to Susceptibility to Field Inoculations with Phymatotrichum Root Rot. WALTER N. EZEKIEL. While under field conditions *Phymatotrichum* root rot is rarely seen on cotton plants less than several months old, successful inoculations in the laboratory of seedlings only a few days old have suggested that this apparent resistance of younger plants might be merely lack of opportunity for infection. An experiment to test this was set up at College Station, Texas, with 50 small plots arranged as 2 Latin squares. Cotton was planted at intervals, and the plants inoculated on July 28, 1939. The final notes 3 months later showed,

Age of plants when inoculated, weeks:	14	10	6	3	0
Plants succumbing to root rot, per cent:	71.1	60.4	42.4	16.6	2.9

The last planting was made over inoculum placed in the furrows. A duplicate final planting adjoining the oldest plants, to allow spread from these to the seedlings, suffered no additional loss. Uninoculated check plots of the various plantings remained almost free of root rot. It is concluded that absence of symptomatic root rot on younger plants under field conditions is evidence of some inherent differences between the older and younger plants, rather than simply a matter of accidental escape.

Field Tests of the Resistance of Cotton to Phymatotrichum omnivorum. G. W. GOLD-SMITH and ELIZABETH J. MOORE. By means of selection based on the growth rates of the fungus, *Phymatotrichum omnivorum*, resistance of cotton to root rot has been tested in the field during the past 2 seasons. Selection has produced a definite increase in resistance. Resistance in some degree exists in many varieties of cotton. If these various degrees of resistance are evaluated by laboratory tests, selection results in a general average increase of this character, but this effect varies in degree with different varieties, among which Native Hopi is most promising. This method of selection and testing in the field produces maximum results when F_2 hybrids are employed.

Effectiveness of Organic Manures in Controlling Cotton Root Rot on Various Soil Types. C. J. KING. The deep application of organic manures in alternate root-rot-infested plots on 3 soil types, Gila fine sandy loam, McClelland clay loam, and Mojave sandy loam, was effective in reducing mortality of cotton 20, 33, and 29 per cent, respec-

tively, below that on unmanured plots in from 3 to 5 years. Plant mortality was delayed by treatment, so that a larger proportion of the crop matured. In two experiments, increased yields were adequate to cover treatment cost. Pure-culture inoculation of every fifth cotton plant in rows planted over heavy applications of cotton-seed meal mixed with hulls, and alfalfa meal, were less effective than inoculations made on nonfertilized rows and only 60 per cent as many plants were killed by subsequent spread of the disease. Pure-culture inoculations of the root-rot fungus on cotton plants in iron drums planted over heavy applications of cotton seed meal, alfalfa meal, and organo-mulch were less effective than inoculations made on plants in nonfertilized drums. Only 40 per cent as many plants were killed by the subsequent spread. From the results in these and other tests in which manures, fertilizers, and crop residues were used in root-rot-infested areas, it appears that organic materials suitable as nutrients for the predominant types of soil microflora, when applied deeply and in concentrated form, will have an inhibitory effect on the root-rot fungus.

Cotton-seed Dusting in Relation to Control of Seedling Infection of Rhizoctonia in the Soil. S. G. LEHMAN. A commercial dust preparation containing 5 per cent of ethyl mercury phosphate was tested as a protectant to cotton seed and seedlings against *Rhizoctonia solani* in the soil. Dusted and nondusted seed lots were planted in the greenhouse in soil that had been steamed and subsequently inoculated with *Rhizoctonia*. Suitable controls were run in steamed, noninoculated soil. Dusting the seed increased emergence of seedlings from inoculated soil significantly more than from noninoculated soil when the seed were planted soon after adding *Rhizoctonia* to the soil, but not when several weeks had elapsed between the time of soil inoculation and seeding. The percentage of seedlings that lived and of those that remained disease-free, however, was not relatively greater by a statistically significant amount on inoculated than on noninoculated soil. The dust applied to the seed before planting gave little or no protection against seedling damping-off by *Rhizoctonia*.

Microorganisms Associated with Cotton-boll Rots in 1939. PAUL R. MILLER. A cooperative survey was conducted in 1939, as in 1938, to obtain information regarding prevalence and relative distribution of microorganisms associated with cotton boll rot. This study confirmed last year's findings that *Glomerella gossypii* was the most widespread and frequent organism encountered. The anthracnose organism was found in 82.3 per cent of the 300 fields from the 13 States surveyed, or in 90.1 per cent of the fields in 11 States, if Texas and Oklahoma are not considered. It was found on a much smaller percentage of the 2959 individual bolls cultured from Texas, Oklahoma, Louisiana, and Arkansas than from States farther east, .0, 1.7, 3.8, and 5.1, respectively, as against 33.2, 34.7, 44.4, and 47 in Mississippi, Alabama, Georgia, and North Carolina, respectively. Anthracnose was detected in none of the 22 samples collected beyond a line approximately 150 miles west of the eastern border of Texas and Oklahoma. No such demarcation could have been predicted from observations of symptoms of diseases as expressed on cotton bolls in these two regions. It would seem that *Glomerella gossypii* in the eastern region either usually initiates green, water-soaked spots, indistinguishable from those caused by the bacteria, or else it occurs in the lesions caused by *Bacterium malvacearum*. Some evidence is presented indicating that the interesting anthracnose distribution may be due to differential humidity conditions. As in 1938, the other boll rot organisms occurring most frequently were *Alternaria* spp., *Fusarium moniliforme*, and other species of *Fusarium*. Other organisms isolated occasionally were: *Diplodia gossypina*, bacteria, and species of *Aspergillus*, *Rhizopus*, *Penicillium*, and *Spicaria*.

Fusarium-wilt Resistance of New Strains and Hybrid Cottons in Louisiana in 1939. D. C. NEAL and H. B. BROWN. The outstanding wilt-resistant cottons in the 1939 test were, in the order named, Delfos 925-425, Dixie Triumph 06-366, Deltapine 12, Dixie Triumph 85, Dixie Triumph 62 x D. & P. L. 10-44-531-62, and Miller 610. The infection ranged from 0.4 per cent for the highly resistant Delfos to 87 per cent for the highly susceptible Half and Half. The position of the leading wilt-resistant varieties as to yield were Deltapine 12, Dixie Triumph 62 x D. & P. L. 10-44-531-62, Dixie Triumph 06-366, Miller 610, Delfos 925-425, and Dixie Triumph 85. It is believed that in a more favorable season the rank in yield of several of the resistant varieties perhaps would have been changed. Stoneville 3-68, notwithstanding its moderate susceptibility (total wilt 25.5 per cent), ranked third in yield of seed cotton. Such behavior, however, has been observed previously with other moderately susceptible but productive varieties. Usually, however, in the case of many varieties when the wilt infection reaches 25 or 30 per cent, many plants are killed, thus lowering the yield significantly. Seed stocks of some of the strains included in this study have been increased and are now available for planting in wilt infested districts. Inquiry should be made to the Louisiana Agricultural Experiment Station.

Experiments on the Control of Downy Mildew of Cucumbers. A. J. FLAKIDAS. Extensive spraying and dusting tests were carried out in the fall of 1939. Bordeaux (4-4-50), Spray Cop (basic copper sulphate), and copper dust (Copox, Copar, and Coprote), with and without adhesive, were used for the bulk of the tests, and smaller plots were sprayed with 4-1.5-50 Bordeaux, yellow copper oxide (Cuprocide 54Y), and phenothiazine. The dust gave the best control and caused no injury to the host. In one field the dusted plants outyielded those sprayed with 4-4-50 Bordeaux by 52 per cent. The Spray Cop gave satisfactory control in 3 of the 4 experimental fields, although less than the dust or the 4-4-50 Bordeaux. In one field, where mildew infection started early, Spray Cop gave inadequate control. Spray Cop caused no injury to the host, and in one field where Bordeaux injury was apparent, the Spray Cop plot outyielded the Bordeaux plot by 30 per cent. The 4-4-50 Bordeaux was next to the dust in degree of control. It caused injury to the host, but the degree of injury varied, being fairly severe in some plots and very slight in others. Bordeaux injury appears to be influenced by amount of soil moisture. In fields with continuous irrigation, Bordeaux injury was slight. There was more injury in 1938 with 2.49 in. rainfall (August 15 to September 17) than in 1939 with 4.99 in. for the same period. Yellow copper oxide gave good control, but caused injury, marginal chlorosis of leaves, and defoliation. The same was true of the 4-1.5-50 Bordeaux. Phenothiazine gave very little control.

The Relation of Nitrogen Fertilization of the Peach on the Control of Bacterium pruni. R. F. POOLE. The bacterial spot of leaf, twig, and fruit of the peach caused by *Bacterium pruni* develops most seriously, and almost entirely, on the peach growing on light sandy soils of the Norfolk type. It is rarely seen on Cecil clay and other heavy soils. Since nutrient deficiency diseases are severe on many crops on the light sandy soils, it was assumed that the nutritional concepts had some bearing on the prevalence of the disease caused by *Bacterium pruni*. After showing that potash and magnesium did not satisfactorily control the disease, studies on the effects of nitrogen on the condition of growth and disease control were conducted on Elberta trees. Starting in July, immediately after harvest, 1 lb. of nitrate of soda was applied every 2 weeks until 6 lb. were applied to 12-year trees. The results were positive. The treated trees maintained dark green foliage until frost and were slightly infected only. The foliage on nontreated trees turned various shades, such as yellow, purplish, and red. They were also severely infected. Defoliation was severe by midsummer. Many of the inner twigs were defoliated and dead before autumn. The fruit bud formation on treated trees is much superior to those on nontreated trees. A quick chemical test of leaves from treated trees showed much greater concentrations of the so-called minor elements, such as magnesium, calcium, iron, potash, and manganese, than those from nontreated trees. It is significant that nitrogen distribution is important on the sandy soils and that the needs of the plant are not satisfied by a few applications. No claim is made that nitrogen alone is responsible for the control of bacterial spot or that the economical application was found in these studies.

Further Studies on the Control of Cercospora Leaf Spots of Peanuts With Various Dusts and Sprays. LUTHER SHAW. Further studies in 1939 on the control of *Cercospora* leaf spots of peanuts have shown that the incubation period for infection of peanut plants with *Cercospora* fungi, under field conditions in North Carolina, ranges from approximately 16 to 21 days. In correlation with this fact it was found in the experiments conducted in 1939 that the maintenance of a 2-week interval between fungicidal applications gave a much greater reduction in *Cercospora* infections on peanut leaves than did applications made at 3- and 4-week intervals. In experiments on the timing of fungicidal applications it was found that 2 applications of sulphur dust made early in the season (July 17, 28) gave an increase in the yield of peanuts almost equal to 4 applications made late (July 28, August 9, 23, Sept. 7). The increase in hay yield from 2 early applications was approximately equal to that from 3 late applications. Six applications depressed peanut yields under those from 5 applications but increased hay yields. In comparative tests various copper and sulphur materials used on both sprays and dusts gave increased yields of peanuts ranging from 408 lb. to 1000 lb. per acre. Leaf-spot control in the 1939 tests was of approximately the same magnitude as found in the 1938 tests, previously reported.

Results of Four Years of Extension Work on Cotton-seed Treatment in North Carolina. LUTHER SHAW. Treating cotton seed with 2% ethyl mercury chloride dust (2% Ceresan) for damping-off control has been actively promoted by the Cooperative-Extension Service in North Carolina for the last 4 years (1936 to 1939, inclusive). During this period a total of 251 result demonstrations have been conducted under practical farm conditions at various points in the State. At each demonstration, treated (3 oz. of 2% Ceresan per 1 bu. of seed) and nontreated seed from the same source were planted under similar field conditions and compared with respect to (a) seedling emergence, (b) kill of

seedlings by damping-off organisms, (c) development of sore shin on surviving seedlings, (d) survival of plants to maturity, and (e) yield of seed cotton. Averaged results of these demonstrations show that the treated seed when compared to the nontreated seed gave (a) 41 per cent increase in seedling emergence, (b) 220 per cent decrease in kill of plants by damping-off organisms, (c) 275 per cent decrease in development of sore shin on surviving seedlings, (d) 33 per cent increase in the number of matured plants, and (e) 12 per cent increase in the yield of seed cotton.

An Occurrence on Cotton of Black Root Rot Caused by Thielaviopsis basicola. C. D. SHERBAKOFF. The writer reports the finding of *Thielaviopsis basicola* on a few cotton seedlings grown in greenhouse, in soil brought in from a field where even *Fusarium* wilt-resistant varieties of cotton went down apparently from a combination of wilt and meadow nematode. The fungus was found also on a few cotton plants grown in the greenhouse, in washed river sand. The inoculation of 12 varieties of cotton with a culture of the fungus (obtained from the diseased seedlings first found), in the greenhouse, in washed river sand, resulted in a black rot of the main root of a large proportion of the seedlings in all cotton varieties tested.

Pathogenicity Tests Conducted in 1939-1940 on Different Isolates of Fusarium vasinfectum. C. D. SHERBAKOFF. The results of 3 series of artificial inoculations of 6 varieties of cotton with a large number of different, monospore isolates of *Fusarium vasinfectum*, and one each of *F. lycopersici* and *F. oxysporum* v. *nicotinae* are here reported. Two isolates of *F. vasinfectum* and the 2 last-named fungi produced no cotton wilt. Some of the isolates of *F. vasinfectum* showed apparently specific differences in the degree of pathogenicity on different varieties of cotton. The results obtained, however, are often inconsistent and, therefore, inconclusive. It is suggested that the inconsistencies might be due to the use of cultures and varieties not sufficiently homozygous in their pathogenicity-susceptibility factors, to the use of selected reisolations of the fungus, and to the small numbers of plants used in each unit of the test.

Storage Tests With Cottonseed. D. M. SIMPSON. The results obtained for the first 2 years of the cooperative seed-storage experiment, set up in 1938, are herein reported. Preliminary findings suggest that certain practical recommendations concerning cottonseed storage may be tentatively offered. (a) Seeds having in excess of 12 per cent moisture should not be placed in ordinary storage unless provision is made for prompt aeration and drying; (b) it seems probable that air-dried seeds may be stored safely for 12 to 18 months in ordinary dry storage at any location in the Cotton-belt; (c) for storage of more than 2 years' duration, seeds should be dried to 8 per cent moisture or less and placed in tight containers to prevent reabsorption of moisture from the atmosphere; (d) seeds containing less than 8 per cent moisture require no aeration and may be kept in air-tight containers for many years without loss of viability; and (e) seeds should be stored in a cool place.

A Regional Study of the Relationship of Potash Treatments to the Development of Cotton Wilt Under Widely Varying Conditions of Soil and Environment. A. L. SMITH. A regional cooperative investigation was initiated in 1937 at 13 locations in 9 States to be conducted for 3 seasons to determine effect of potash level, 0, 32, and 64 pounds of K_2O per acre, on wilt and yield of 12 varieties of cotton, varying widely in wilt resistance. Analysis of data for the third is not yet available. A combined summary of all locations indicates a significant reduction in total infected plants at both the 32- and 64-lb. applications. A significant increase in yield is shown at the 32-lb. level with a further but not statistically significant increase at the 64-lb. level. Response in yield and wilt reduction varies greatly with individual locations. At most locations, yield response is roughly proportional to reduction in wilt. Response of susceptible varieties to potash treatments is similar in direction to that of Resistant ones, although there is some indication of variety-potash interaction. The yield response of susceptible varieties, however, is insufficient to overcome losses occasioned by wilt; consequently, varietal resistance is more important to maintenance of yield. Comparison of cotton harvested from healthy and diseased plants indicates reduced staple length, reduced seed weight, and increased lint percentage from diseased material. Relative differences in varietal susceptibility to wilt at different locations appears to be partly attributable to varying nematode infestations and differences in varietal reaction to a combination of wilt and nematodes. Field evidence in support of the belief that this condition may be due to the occurrence of variation and host specialization of the pathogen at different locations is weak.

Notes on the Pathogenic Action of Phymatotrichum omnivorum. G. M. WATKINS and M. O. WATKINS. Juices were expressed from roots of cotton seedlings that had been thoroughly decayed by the mycelium of *Phymatotrichum omnivorum* (Shear) Duggar, and

also from sclerotia of the fungus. A portion of the liquid from each source was applied as drops directly upon the surfaces of healthy seedling roots of cotton; another similar portion was heated to the boiling point for 1 hr. and, when cool, applied similarly to other seedling roots. The nonheated liquid from each source caused swelling, distortion, and increased capacity to retain dyes in walls of the exposed cells, followed by collapse of the tissues, producing a dark-staining layer of disorganized cells. The heated liquid produced no appreciable changes in the cell walls, but caused considerable disorganization of protoplasts.

Fungi Found on Diseased Cotton Seedlings From Thirteen States Surveyed in 1939. R. WEINDLING. Diseased cotton seedlings collected through the efforts of the Plant Disease Survey cooperating with State and federal pathologists were cultured in order to determine the relative frequency and distribution of fungi connected with the damping-off disease. As in 1938, *Glomerella gossypii* was isolated much more frequently than *Rhizoctonia solani*. Of the 11,090 seedlings examined, 6,300 collected from 89.4% of the fields showed *G. gossypii*, while *Rh. solani* was present only on 153 seedlings from 13.2 per cent of the fields. *G. gossypii* was the predominant organism in all states east of Texas and Oklahoma, but it was not in evidence on any of the seedlings obtained from these two states. Other fungi which are not considered as primary causative agents of the disease are listed here in descending order according to their frequency of occurrence: *Fusarium moniliforme*, *Fusarium* spp. (including *F. vasinfectum*), *Alternaria* spp., *Rhizoctonia bataticola*, *Diplodia gossypina*, *Penicillium* spp., *Aspergillus* spp., and *Chaetomium* spp.

Root Rot of Austrian Winter Peas and Vetches. J. L. WEIMER. Austrian Winter peas (*Pisum arvense* L.) and vetches (*Vicia* sp.) constitute the winter cover crops planted over considerable areas of the southern United States. Both peas and vetch often are more or less severely affected by a root rot. The roots may show early stages of decay as early as December, but the plants usually do not die before late winter or early spring. The losses vary greatly from year to year, being greatest during a wet winter and spring. The tops of pea plants become yellowish, dwarfed and gradually die. Vetch plants respond in a similar manner, but the affected parts and often the tops show more red color. The degree of color varies in the different vetches, the brick red in Monantha often being very striking. The affected roots contain oospores of *Aphanomyces*, probably *A. euteiches* Drechs., and this fungus is thought to be a major factor in initiating this disease. *Pythium graminicolum* Subr., *P. irregulare* Buisman, *Rhizoctonia* sp., *Fusarium* sp., bacteria, and nematodes are associated in many of the roots. The first three fungi are capable of causing more or less root decay under certain conditions. Suggestions for control include proper fertilization, good drainage, and as long a rotation as practical. Hairy, Smooth, Light-seeded Hungarian, and Hungarian vetches and *Vicia hybrida* are fairly resistant, although not immune. Wooly Pod, Monantha and Common vetches are susceptible. No resistant variety of peas has been seen. (Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Georgia Agricultural Experiment Station.)

Some Observations on the Development of Root-knot Nematode Diseases in Virginia. S. B. FENNE. In Caroline County, Va., it was found in the past year that certain fields of Nancy Hall sweet potatoes on at least 2 farms were heavily infested with root knot. In working with the growers in an attempt to give them a rotation that will reduce the nematode population, the supposedly resistant Laredo soybeans were grown. Just before cutting time these beans were examined and found heavily infested with root knot. Some of the roots were an inch or more in diameter. The second situation exists in the 4 counties of Northern Neck, Virginia, where tomato plants from a state farther south have been used for the last 2 or 3 years. Some of the plants obtained last year were badly infested with root knot, resulting in a decreased yield of from 15 to 75 per cent in some cases.

Some Suggestions for Quick Testing of Nematode Resistance in Plant-breeding Programs. G. H. GODFREY. Reference is made to an earlier paper, (Some Technique used in the study of the root-knot nematode, *Heterodera radicicola*. Phytopath. 21 (3), 323-329, March, 1931), with the suggestion that certain phases of the technique described therein might be used to advantage to obtain quick readings on root-knot-nematode resistance in plant-breeding programs. Careful application of the method would eliminate the complication and delay occasioned by chance "escape" from infestation on the part of a few plants in a progeny population.

Resistance to Root-knot Nematode in Nicotiana. E. E. CLAYTON. Studies on the soil carry-over of nematodes, following cropping with peanuts, resistant soybeans and cowpeas, cotton, corn, etc., have shown that only a few crops do actually starve out the nema-

todes. Even Brabham peas and corn are sufficiently parasitized to carry over large nematode populations. There are important differences thus between resistant and immune plants. To provide a sound basis for tobacco breeding work, studies have been made of the occurrence of nematode resistance in the cultivated *N. tabacum* and other *Nicotiana* species. Within *N. tabacum* varying degrees of resistance were found. It has been possible (1) to eliminate certain lines, like White Honduras, where nematode resistance is linked closely with undesirable growth characters; (2) to eliminate many lines with intermediate types of resistance; and (3) to locate, and establish, with repeated selfing, apparently homozygous lines of *N. tabacum* with a marked degree of nematode resistance. Studies of other *Nicotiana* species have shown that many are highly susceptible, thus *bigelovii*, *maritima*, *langsдорffii*, *glutinosa* and *goodspeedii*. Some were highly resistant or immune. These include *longiflora*, *megalosiphon*, *repanda*, *nudicaulis*, and *nesophila*. Such species provide genetic material far superior, from the viewpoint of resistance, to anything discovered within *N. tabacum*. The object of this work is (1) to produce locally well-adapted commercial tobacco varieties that are nematode-resistant and (2) to develop basic stocks available to anyone undertaking a tobacco breeding project. We hope to be able to eliminate down to a single source of immunity that is conditioned by one or two genes. The need for such immune, easily handled, basic, breeding materials has led us to give much attention to the other *Nicotiana* species, rather than to confine our work to the resistance occurring in cultivated tobaccos.

Control of Root Knot in Florida Cigar-wrapper Tobacco Fields. RANDALL R. KINCAD. An experiment was begun at Quincy, Florida, in the fall of 1937 on 1 acre of shade field where 15 consecutive crops of cigar-wrapper tobacco had been grown. The soil was heavily infested with root-knot nematodes. Treatments were in quadruplicate plots having a net area of about 1/35 acre each. Crops of tobacco were grown each year, and harvested, cured, sweated, and graded according to usual commercial practices. All plots were plowed in January and treated alike until the end of the crop season in July. Frequent plowing from July till January kept the first series in clean fallow. In the second series the tobacco roots were removed from the field and the soil kept in clean fallow. In the third series clean fallow was followed by a cover crop of oats from October to January. All three produced good crops in both seasons, with no significant differences. The fourth series, in which an unsuccessful attempt was made to grow sorghum in rows from July to November, followed by fallow, showed a significant increase in root knot and a reduction in yield. The fifth series, where native vegetation, mostly grasses, grew from July to January, showed a further small reduction in yield because of an adverse effect of the cover crop of native grasses rather than an increase in nematode population. The tobacco grade index for the 5 treatments was essentially the same.

Comparison of Crop Rotation and Fallowing as Methods on Control for Root Knot of Cotton Under Irrigation. C. J. KING. A cotton-alfalfa rotation, practiced for 20 years on an area affected by root-knot nematodes, indicated that satisfactory yields of American-Egyptian cotton could be maintained under conditions of the experiment, if the intervals between alfalfa were not greater than 2 years. It appeared that high yields of upland cotton could be maintained for several years without return of the area to alfalfa. The practise of clean fallow combined with deep tillage in summer for 3 years was found effective in eliminating nematodes as a damaging factor to American-Egyptian cotton for 1 year, and no serious damage resulted the second year, although some of the area had been reinfested. One year of alfalfa showed advantages over 1 year of fallow in improving production of American-Egyptian cotton in an area heavily infested with root-knot nematodes. On the basis of the information obtained in these experiments, alfalfa rotation would appear to be equally as effective and much more profitable than fallowing as a practical method for the control of root-knot nematodes affecting cotton.

Recent Root-Knot Damage in Potatoes. JOHN T. MIDDLETON. Potatoes harvested from a 40-acre field in Southern California were severely infested with nematodes, with a loss of about 40 per cent of the crop. In an adjoining field of 10 acres, there was less than 1 per cent nematode infestation. Neither field had ever produced potatoes prior to this time. The 40-acre block had been in peaches the year before. The peaches affected with nematodes had been cut down and pulled out and the ground fallowed prior to planting the potato crop. The 10-acre block had been under cultivation for about 8 years, having produced only grain crops. A 20-acre block of potatoes, which yielded only 80 hundred-pound sacks to the acre, as compared with an average of 150 per acre, was found severely infested with nematodes, about 80-90 per cent. This same block has been in potatoes for the past 3 years. Each year the infestation has become progressively worse. If proper cultural practices had been employed the excessive losses could have been appreciably reduced. In general, nematode infestation of potatoes is more pronounced and causes greater damage to the fall crop (July-September).

The Effect of Crop Rotation on the Control of Heterodera marioni on Norfolk Sandy Loam. K. J. SHAW. On land heavily infested with *Heterodera marioni*, rotation experiments have been conducted during the past 3 years. Two types of plots are used: (a) Inclosure units, 12×24 ft., and (b) 1/20-acre field plots.

(a) *Inclosure Units.* Tobacco following velvet beans, bare fallow, oats and bare fallow and crotonaria (*Spectabilis*) showed less than 10 per cent severe root knot. Tobacco following peanuts showed 14 per cent; soybeans (Laredo) 69.8; weeds (with crabgrass) 75.8; oats and weeds 77.5; and corn, cotton, sweet potatoes and tobacco 100 per cent severe root knot. Tobacco following 2 years' bare fallow showed no severe root knot, following 2 years' herdsgrass (Red top) 8.3; 2 years' lespedeza (Tenn. 76) 83.7; and tobacco 100 per cent severe root knot.

(b) *Field Plots.* Based on 2-year averages, tobacco following peanuts showed 11 per cent severe root knot, weeds 35.5, oats and weeds 43.5, cotton 44.3, corn 67.3, and tobacco 93.3 per cent.

In the 3-year rotations, based on 1 year's results, cotton-peanuts-tobacco, peanuts, oats, and weeds-tobacco and weeds-weeds, tobacco showed less than 10 per cent severe root knot. Peanuts-cotton-tobacco and corn-oats and weeds-tobacco showed less than 25 per cent. Corn-cotton-tobacco showed 28 per cent, cotton-weeds-tobacco 45 per cent, and continuous tobacco 93 per cent. Data also were obtained on yield and value per acre but these do not necessarily correspond to the amount of severe root knot.

Distribution and Relation of Meadow Nematode, Pratylenchus pratensis, to Fusarium Wilt of Cotton in Georgia. A. L. SMITH. The widespread distribution of the meadow nematode in wilt soils both in the Piedmont and Coastal Plain suggests its probable important relationship to *Fusarium* wilt of cotton. The meadow nematode was the predominant species in several fields in which ordinarily resistant varieties showed considerable wilting. A complete killing of all plants by wilt in other limited areas in fields of susceptible and semiresistant varieties was likewise attributed to the heavy meadow nematode population. A detailed study of nematode populations in a heavily wilt-infested field near Cuthbert showed the meadow nematode to be the predominant species. There was also a heavy infestation of a form which Steiner has identified as representing the new species being described from Hawaii by Linford. The importance of the latter species in relation to wilt has not been determined. The root-knot nematode was found relatively infrequent in the Cuthbert plot.

On the Occurrence of the Banana Nematode (Pratylenchus musicola) in the United States. G. STEINER. Heavy infestations of the banana nematode, a close relative of the meadow nematode, have been found on roots of fig, olive, and black walnut received from southern California. Black lesions develop until the root cortex is destroyed; the healthy distal roots of fig may even be amputated by destruction of the axial cylinder. This nematode, also, has been found recently killing boxwood plants in Virginia.

The Root-knot Nematode Attacking Stems and Leaves of Plants. G. STEINER. Large numbers of root-knot larvae were observed entering the cotyledons, stems, and leaves of beans germinating in heavily infested soil. Many of the young plants were killed by this extreme infestation before the nematodes could multiply, and thus acted as a trap crop; the next planting of beans showed only slight infestation.

Further Observations on the Nematode-fusarium-wilt Experiments at Lumberton, North Carolina. A. L. TAYLOR, H. D. BARKER, P. H. KIME. In 1939 the set-up was similar to that of 1938 reported on at the New Orleans meeting (Phytopath. 29: 752). Results for 1939 were not materially different. The carbon-bisulphide treatment controlled nematodes sufficiently to enable various wilt-resistant and wilt-tolerant varieties of cotton to become established and make satisfactory growth. Only a moderate amount of wilt developed in treated plots. In nontreated plots few plants had not been killed by middle of July. Sea Island was the only variety to stand up in the nontreated plots. It was not killed, but was severely stunted. Root knot was abundant and many secondary roots were killed by the meadow nematode. It is encouraging that Sea Island survived and sent out new secondary rootlets, although it made poor growth. There was no conclusive evidence for varietal differences with respect to resistance either to the root-knot nematode or the meadow nematode. Sea Island survived and there was a slight indication that such wilt-resistant varieties as Coker's 4 to 1 and Cook 307 survived somewhat longer than such less resistant varieties as Delta Pine 12, Mexican Big Boll, and Miller. These differences were attributed to wilt-resistance characteristics rather than to nematode resistance. The differences between carbon-bisulphide-treated and nontreated plots, however, suggested that abundance of nematodes influenced infection by the wilt organism. Sea Island thus has some characteristic that even the most wilt-resistant upland varieties do not possess. Hence further experiments with Sea Island-upland hybrids and back crosses may be profitable producers of a combined wilt and nematode resistance.

Suggestions Arising From an Analysis of Plants Reported Resistant or Tolerant Toward Root-knot-nematode Infestation. JOCELYN TYLER. In assembling data for a compilation, now in press, on the above subject, there was found a surprising dearth of accurate, first-hand information on certain plants. Careful field records are needed on the degree of infestation or resistance to root knot found in specific grasses and other plants commonly considered resistant, and in other plants that might possibly be found resistant or tolerant. Gross symptoms, either above ground or below, are not always dependable evidence. A proposed outline for reports includes the species and variety of the plant or root stock, crop and nematode history of the land, indications of unevenly distributed root-knot populations, the season of observation, and detailed descriptions of the amount and character of infestation and its effect on the plants.

Chemical Treatment of Soil to Control the Root-knot Nematode. P. A. YOUNG. Several chemicals were tested to determine their value in controlling *Heterodera marioni* in sandy soil. Extensive tests showed that chloropicrin, injected into the soil at rates of 300 to 600 lb. per acre, usually controlled 90 to 100 per cent of the root-knot nematodes. Likewise, injection of 1000 to 3000 lb. of carbon bisulphide per acre controlled all or most of the root-knot nematodes in numerous tests. These 2 chemicals controlled the nematodes efficiently only when the soil was covered during 4 or more days with paper coated with animal glue, casein, or vegetable paste. Comparative tests showed that wetting the top 2 to 3 in. of soil with water immediately after injecting these chemicals confined the chemicals in the soil so well that the water cover was nearly as efficient as the glue-coated paper in retaining the chloropicrin gas. Fumigating soil with chloropicrin at the rate of 10 cc. per cu. ft. of soil controlled soil-borne parasites. Formaldehyde, cyanamid, and sodium hydroxide were not effective in decreasing the abundance of nematodes in the soil. In the control of nematodes, watermelons were used as test plants in the treated and nontreated soil to ascertain the effectiveness of the treatment. Usually 75 to 100 per cent of the watermelon plants had root knots when grown in the nontreated (check) soil. Evidence was secured that the pocket gopher carried root-knot nematodes from nontreated soil into disinfected soil of a hot bed.

PLANT NEMATODE COUNCIL

At the meeting of the Third National Plant Nematode Conference held in Birmingham, Alabama, on the afternoon of February 9, 1940, those present, numbering about thirty-five, unanimously approved recommendations for the formation of a permanent organization, presented by the committee which had arranged for the conference.

The adopted recommendation is as follows:

It is the recommendation of the present committee that this organization be made permanent.

That it shall be known as the Plant Nematode Council.

That its purpose shall be to promote research on plant nematode problems and to facilitate mutual assistance among the workers.

That membership shall be given to all interested in plant nematode research.

That the existing committee shall become the Executive Committee; that it shall have power to increase or otherwise change its membership, and to select a chairman and a secretary; and that it shall prepare and submit to the membership a program of suggested activities.

The Executive Committee met immediately after the conference and elected Dean H. Harold Hume, University of Florida, chairman, and Howard P. Barss, U. S. Department of Agriculture, as secretary. G. H. Godfrey of Texas, R. J. Haskell of the U. S. Extension Service, H. D. Barker of the U. S. Bureau of Plant Industry, and B. L. Wade of the Southeastern Regional Vegetable Breeding Laboratory, were added to the Executive Committee which already consisted of H. Harold Hume, Florida, H. P. Stuckey, Georgia, R. F. Poole, North Carolina, C. D. Sherbakoff, Tennessee, S. A. Wingard, Virginia, K. C. Barrons, Michigan, and G. Steiner and E. E. Clayton, U. S. Department of Agriculture.

It was tentatively agreed to hold another general conference of all interested in plant nematode research the following year, preferably in conjunction with the Association of Southern Agricultural Workers. It was decided that one of the first steps of the Council should be to work out, if possible, a means whereby workers in various states could cooperate in more systematic testing of varieties of crop plants, forage plants, erosion control plants, as well as other kinds of plants for resistance to nematodes.

HOWARD P. BARSS, *Secretary*



THE PATHOGEN OF FILBERT BACTERIOSIS COMPARED WITH PHYTOMONAS JUGLANDIS, THE CAUSE OF WALNUT BLIGHT¹

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H. N. GROSS, AND H. P. BARSS

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INTRODUCTION

Bacteriosis of filberts, commonly known as filbert blight, is the most serious and, so far as is now known, the only infectious disease of filberts (*Corylus avellana* L. and *C. maxima* Mill.) of any economic importance in the Pacific Northwest. It was first brought to the attention of H. P. Barss late in 1913. Microscopical examinations of diseased tissues disclosed the presence of bacteria, and repeated isolations consistently yielded a bacterium of a specific type. The disease was reproduced on filbert by inoculations with pure cultures of this bacterium, and the same organism was reisolated from the lesions produced, thereby definitely establishing its causal relationship to the disease.²

A preliminary report describing the malady and covering early investigations of its cause and life history was published by one of the writers in 1915 (2). Although a number of publications (3, 15, 16, 17, 18, 19, 20) on the symptomatology, life history, and control of this disease have since appeared, no detailed studies of the causal organism have ever been published. The striking similarity in appearance of the filbert and the walnut bacteriosis organisms on the more common bacteriological media prompted the question at the very outset as to relationship between these two pathogens. The characteristics of the filbert-blight pathogen are discussed in detail in the present paper and compared with those of *Phytomonas juglandis* (Pierce) Bergey *et al.*, the cause of the common bacteriosis of the Persian (English) walnut (*Juglans regia* L.).

THE PATHOGEN

Source and Number of Isolates

In all, 26 different isolates of the filbert-blight pathogen from widely scattered filbert orchards in Oregon and Washington were used in the pres-

¹ Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station. Published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 317. Grateful acknowledgments are made to Dr. J. A. Pinckard Jr., R. H. McBee and others who assisted in various aspects of these studies.

² The only other reference found by the authors relating to bacteria associated with *Corylus* is the report by Brzezinski in 1903 (5) of *Bacterium coryli* on hazel in Europe. This author gave no adequate description and reported no tests for pathogenicity, for which reason the name is listed in the 1939 Edition of Bergey's Manual, p. 217 (4), among those excluded because of inadequate description or unproved pathogenicity. Brzezinski reports that the organism is the same culturally and morphologically as *B. mali*, an organism described by him as grayish white on agar.

ent studies. While not all of these were used throughout, at least 4 different isolates from widely scattered locations were applied in each phase of the investigation.

A total of 17 different isolates of *Phytomonas juglandis* from widely scattered walnut orchards in Oregon, Washington, and California were used at one time or another in the various phases of this investigation. In most of the comparative studies 3 isolates from walnut from widely scattered locations were used.

Method of Purifying Cultures

Twelve to 24-hour-old Difco beef-extract-dextrose broth cultures of each isolate were shaken thoroughly and three successive series of dilution plates were made in the conventional manner, after which sub-cultures were made from single colonies. This method, as McNew (14) states, gives a very high percentage of colonies which are single-cell cultures.

Morphology and Staining Reactions

Methods. Morphological characteristics of the isolates were determined from 48-hour-old cultures grown at 28° C. on Difco beef-extract-dextrose nutrient agar, adjusted to pH 7.4. For form and size, negative demonstrations from smears prepared with 1 per cent nigrosine were used. Hiss' method was employed for demonstrating capsules, and Cesares-Gil's flagella stain was used to determine the number and position of flagella. Gram reaction was determined according to Burke's modification of Gram's stain. Dorner's method was used as a test for spores, and for acid-fast properties Ziehl-Nielsen's method was employed.

Morphological Characteristics. A detailed study of four filbert blight

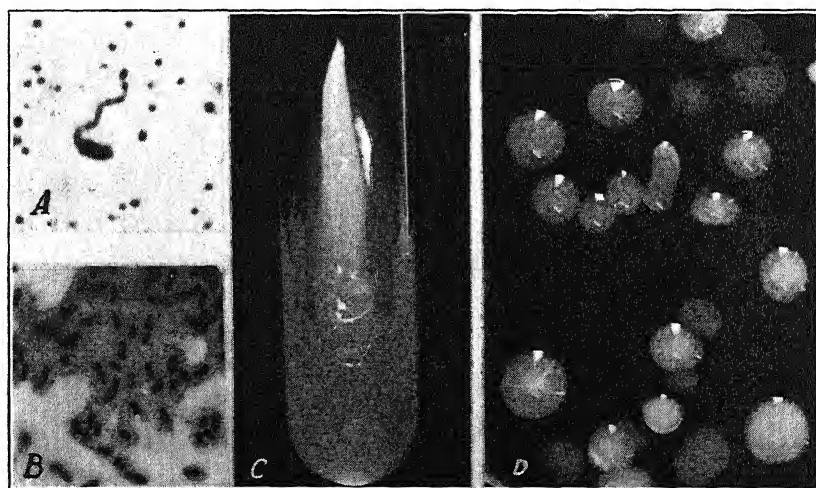


FIG. 1. A and B. Photomicrographs of *Phytomonas corylina*: A, the single polar flagellum, $\times 2300$; B, capsule about organism, $\times 1475$. C and D. Ten-day-old potato-dextrose-agar cultures of the same organism: C, streak culture; D, plate culture. $\times 1$.

isolates from filbert, supported by numerous observations of many others, showed the filbert blight pathogen to be a short rod with rounded ends, arranged singly or in pairs, and occasionally in short chains. The cells measure from 1.1 to 3.8 μ by 0.5 to 0.7 μ . The organism is motile by one polar flagellum. (Fig. 1, A.) It is Gram-negative and is not acid fast. On nutrient-dextrose agar the organism is heavily capsulated. (Fig. 1, B.) No endospores are produced. It stains readily with gentian violet and carbol fuchsin, but only very lightly with methylene blue. In respect to these characteristics, no difference between the 4 isolates studied was noted. Three isolates of *Phytophthora juglandis* from walnut studied comparatively had similar morphological characteristics. Smith (21), who has made the most detailed study of the walnut-blight pathogen to date, reported *P. juglandis* to measure 1.5 to 3.01 μ by 0.3 to 0.51 μ . The smaller size given by Smith may be attributable to his having used "positive" staining methods; whereas, in the present investigation, the "negative" staining technique was employed. Smith states that *P. juglandis* does not possess a true capsule. The writers found *P. juglandis* definitely capsulated, but capsule formation seemed slightly more abundant in the case of the isolates from filbert. Smith states also that *P. juglandis* is Gram-positive, whereas all filbert and walnut blight isolates used in the present study were found Gram-negative. In the other respects, Smith's description of the morphological characteristics of *P. juglandis* is in agreement with the above.

Cultural Characteristics

Nutrient Dextrose Agar Streak.—Growth of 4 isolates from filbert on Difco beef-extract-dextrose agar at pH 6.6, after 48 hours at 28° C., was moderate, filiform, convex, viscid, glistening, smooth, opaque, odorless and pale lemon-yellow (copper-yellow: No. 1 (23)). Three isolates of *Phytophthora juglandis* from walnut, studied comparatively, showed no significant differences from the filbert isolates in growth on this medium.

Potato-dextrose Agar Streak.—Growth of 4 isolates from filbert on Difco potato-dextrose agar at pH 6.8, after 3 days at an average temperature of 25° C., was abundant, filiform, convex, viscid, glistening, opaque, odorless, and a pale-lemon to light-chrome yellow (amber-yellow: No. 1 (23)) in color (Fig. 1, C). The topography of the growth of all isolates was typically smooth, although a wrinkling or infolding of the surface was noted in some of the isolates after they had been in culture for several months. At room temperature, the bacterial growth covers the entire width of an agar slope at the base in from 10 to 15 days. The behavior of three isolates of the organism from walnut studied comparatively was essentially the same with only insignificant variations between isolates.

On Potato Cylinders.—Growth of four isolates from filbert grown on sterilized potato cylinders after 3 days at 28° C. was moderate, viscid, filiform to echinulate, convex, glistening, smooth, opaque, odorless and deep lemon to chrome-yellow. The medium was unchanged except for the forma-

tion of a partially cleared "fermentation" zone, 2 to 4 mm. in width, just beyond the margin of growth. Tests with Gram's iodine solution showed that this zone was free or nearly so from starch. Three isolates from walnut studied in comparison behaved in a like manner on this medium.

Nutrient Dextrose Agar Plates.—Colonies of all four isolates of the organism from filbert studied became visible on Difco beef-extract-dextrose agar plates in from 2 to 3 days at room temperature, averaging 22° C. After 6 days' growth, the surface colonies of all isolates were circular, smooth, convex, glistening, with entire margins. The colonies imbedded in the medium were lenticular. The internal structure of the surface colonies was homogenous and finely granular. The medium remained unchanged in color. The colonies of all isolates were pale lemon-yellow (coppery-yellow: No. 1 (23)) at the margins and deeper lemon to chrome-yellow at the centers. After 12 days growth, the colonies averaged about 7 mm. in diameter. Three isolates of *Phytomonas juglandis* from walnut studied comparatively showed no essential differences from the foregoing in growth on this medium.

Potato-dextrose Agar Plates.—Colonies of 4 isolates from filbert on Difco potato-dextrose agar plates became visible at an average temperature of 22° C. in from 2 to 3 days. After 5 to 6 days' growth the surface colonies were circular and those imbedded in the medium, lenticular. The surface colonies of all isolates were viscid, smooth, convex to pulvinate, with entire edges. (Fig. 1, D.) The internal structure was finely granular. In some colonies radial striations occurred at the margins. The colonies were a pale lemon to a light chrome-yellow (amber-yellow: No. 1 (23)). There was no change in the color of the medium except just in advance of the margin of each colony where, because of the hydrolysis of the starch in the medium, there was a partly clear zone. This zone became very noticeable when the plates were flooded with Gram's iodine solution. At the end of 9 days the colonies averaged approximately 9 mm. in diameter. Three isolates of *Phytomonas juglandis* from walnut studied comparatively gave comparable results on this medium.

Gelatin Stab.—Growth of 4 isolates from filbert in Difco nutrient-gelatin "stabs" at pH 6.6 became visible after 24 hours at an average temperature of 22° C. In all cases liquefaction began at the surface in 1 to 2 days. The liquefied portion was at first infundibuliform but soon became stratiform. In 10 days the upper half of the medium had become liquefied. The liquefied portion was slightly turbid and a pale yellow sediment was evident at the bottom. Liquefaction of the lower half of the medium was very slow, requiring from four to six weeks or even longer for 10 cc. of gelatin in a $\frac{5}{8}$ inch test tube to be entirely liquefied. The behavior of three isolates of *Phytomonas juglandis* from walnut studied comparatively was essentially the same on this medium.

Twenty-six isolates from filbert and 8 of *Phytomonas juglandis* were tested by Frazier's gelatin-plate technique (11). After 48 hours' incuba-

tion at 30° C., all cultures gave a positive reaction with tannic acid, indicating a considerable increase in amino-nitrogen.

Other Solid Media.—Nine isolates of the filbert blight pathogen and a like number of *Phytomonas juglandis* were grown comparatively at the same time and under like conditions upon a number of other solid media. Growth of all isolates from walnut and filbert was sparse on Difco prune agar, Difco oat agar, Difco Lima-bean agar, Difco corn-meal agar and Difco bean-pod agar. On Endo-agar, Congo red agar, and Levine's eosine-methylene blue agar growth of all walnut and filbert isolates, while normal in amount, was not sufficiently distinctive to justify detailed description.

Nutrient Broth.—In Difco beef-extract-peptone broth at pH 6.6, all 4 isolates from filbert made a trace of growth after 16 hours at room temperature, averaging 22° C. By 48 hours a moderate clouding of the medium was evident in all cases, but there was no sediment. After 6 days the medium was very turbid and a fragile ring developed where the surface of the medium met the walls of the tube. This ring was at first readily broken up into flocculent particles by agitation. Later it became more cohesive but never developed into a true pellicle. After about 10 days a pale-yellow sediment formed in the medium, which was slightly viscid on agitation. The medium cleared slightly after about 3 weeks, due to a settling of the growth. No odor was at first detectable, but after several weeks there was a slight odor resembling ammonia. The 3 isolates from walnut, studied comparatively, gave similar results in this medium.

Dextrose Broth. In Difco-dextrose broth at a pH of 6.6, all 4 isolates from filbert made a trace of growth after 24 hours at 30° C. After 3 days at 30° C. all cultures showed a moderate clouding of the medium but no sediment. By the third day a fragile ring-like growth formed at the surface, which was easily broken up into flocculent particles by agitation. Later this surface growth became more cohesive but never developed into a true pellicle. An odor resembling ammonia developed after several weeks. Three isolates of *Phytomonas juglandis* from walnut, studied comparatively, gave similar results in this medium.

PHYSIOLOGY

Methods

The recommendations of the Committee on Bacteriological Technique, Society of American Bacteriologists, as given in the Manual of Methods for Pure Culture Study of Bacteria (8), were closely followed by the writers in most of their biochemical studies. The cultures were incubated at room temperature, which averaged 22° C., unless otherwise stated.

Biochemical Characteristics

Relation to Free Oxygen.—All 4 of the isolates from filbert were strongly aerobic as indicated by the fact that when grown in Smith's fermentation tubes in Difco nutrient broth containing 1 per cent dextrose, growth

occurred only in the open arm. Three strains from walnut, studied comparatively, were likewise strongly aerobic.

Nitrate Reduction.—None of the 4 isolates from filbert reduced nitrates to nitrites, nor was any gas formed in a nitrate broth. To test for the reduction of nitrates, Trommsdorf reagent was added to 1-, 2-, 4-, and 7-day old cultures, respectively. The 3 isolates from walnut, studied comparatively, likewise failed to reduce nitrates.

Chromogenesis.—All 26 isolates from filbert cultured during the course of the investigation produced a yellow pigment on Difco nutrient agar and Difco potato-dextrose agar. The color of the growth varied from the paler shades of yellow to deeper yellows, depending on the age of the cultures and on the medium. The prevailing tone is a pale lemon-yellow (amber-yellow: No. 1 (23)). Seventeen strains from walnut, studied comparatively, likewise produced a yellow pigment of the same hue. On certain media both the filbert and walnut-blight isolates varied somewhat in the intensity of the yellow pigment produced, but there was a greater variation between individual isolates within a group than between groups.

Indole Production.—Indole was not produced by any of the 4 isolates from filbert studied by standard procedure (8). To test for indole production, 2-day-old cultures in tryptophane broth were tested with Ehrlich's reagent. Three walnut-blight isolates, studied comparatively, likewise failed to produce indole. Smith (21), using a different procedure, reported a strong but delayed indole reaction at 75° to 80° C. when 2-week-old cultures of *Phytophthora juglandis* in Dunham's solution were tested with 0.02 per cent sodium nitrite and a few drops of concentrated sulphuric acid.

Hydrogen Sulphide.—None of the 4 isolates from filbert produced hydrogen sulphide when grown in Difco lead acetate-agar stabs. Three isolates from walnut, studied comparatively, also failed to produce hydrogen sulphide.

Hydrolysis of Starch.—Tests for starch hydrolyzing ability were made by growing ten filbert isolates on plates of potato-dextrose-agar. A cleared zone, which became very noticeable when the surface of the medium was flooded with Gram's iodine solution, was produced about colonies of all the isolates. Eight isolates from walnut, studied in comparison, likewise hydrolyzed starch.

Digestion of Milk.—The action on milk of 4 isolates from filbert was studied; each produced an enzymatic curd that was slowly digested. Peptonization occurred near the surface of all cultures after 4 to 5 days; it was more pronounced, though not complete after 10 days. Three isolates from walnut, studied comparatively, also digested milk in a similar manner.

Reduction of Litmus.—Four isolates from filbert were studied and found to slowly reduce litmus in litmus milk. Reduction began after 1 to 2 days, but was not complete until 1 to 2 months thereafter. Three walnut blight isolates from walnut, studied comparatively, likewise slowly reduced litmus in litmus milk, as given above.

Selenium Reduction.—Streak cultures of all 26 isolates from filbert on nutrient agar containing selenium dioxide in 1:25,000 concentration became brick-red after a short time, indicating intracellular reduction. Each of the 17 isolates of *Phytophthora juglandis* from walnut, comparatively studied, likewise reduced selenium dioxide. This reaction is regarded by Levine (13) as a better indicator of reducing activity than are organic dyes.

Methyl Red Test.—Cultures of 4 isolates from filbert on Difco M.R.-V.P. medium, adjusted to an initial pH of 6.9, became progressively more alkaline with age. Three isolates from walnut, studied in comparison, likewise became progressively more alkaline on this medium.

Alkali Production.—Alkali (ammonia) is produced by both the filbert and walnut-blight pathogens when grown in a nutrient broth containing peptone as a nitrogen source. Thus, a shift in reaction from an initial pH of 6.8 to a pH of 7.5 occurred after 11 days growth in Difco nutrient broth. Tests with Nessler's reagent were positive, showing that ammonia was formed. Ammonia also was produced abundantly in a 1 per cent solution of peptone in tap water. Although the filbert- and walnut-blight pathogens produce an alkaline reaction in peptone-containing media and in milk, they do not belong to the alkali-forming group of bacteria, as defined by Ayers *et al.* (1). Alkalinity with the filbert- and walnut-blight pathogens, when grown in nutrient broth, is apparently induced by ammonia from amino acid breakdown. Alkaline carbonates formed by the oxidation of organic acid salts also give an alkaline reaction, as shown in table 1. Dowson (9) recently reported that *Phytophthora juglandis* does not produce ammonia in nitrate-peptone broth.

Hydrogen-ion Relations.—Studies of the hydrogen-ion relations of one isolate of the filbert-blight pathogen from filbert and one isolate of *Phytophthora juglandis* from walnut indicate that these two organisms have quite similar hydrogen-ion relations. The filbert-blight pathogen grew at a pH range of from 5.2 to 10.5. It made the most rapid growth, however, from pH 6 to 8. The hydrogen-ion concentration, at which growth in Difco nutrient broth was inhibited, was pH 5 in the acid range and pH 11 in the alkaline range.

The isolate of *Phytophthora juglandis*, studied comparatively, had very similar hydrogen-ion relations with the exception that it did not have quite so low a minimum pH. It did not grow at pH 5.2, but did make a small amount of growth at pH 5.4.

Carbon Metabolism.—A variety of carbon sources are utilizable by both the filbert- and walnut-blight pathogens with the production of acid but no gas. To demonstrate acid production it was found necessary to employ a synthetic medium containing only inorganic nitrogen compounds, as acid production is completely masked in the presence of peptone because of ammonia production. The utilization of carbohydrates by 4 isolates from filbert and 3 from walnut were studied in preliminary investigations with agar slopes made with a synthetic basal medium according to the method of

Burkholder (6). After 10 to 14 days' incubation at 28° C., it was observed that both the walnut- and filbert-blight isolates produced acid from dextrose, but the former grew more slowly; with sucrose this same difference in rate of growth prevailed and, in addition, only the isolates from filbert produced acid. Consistent differences with other carbon sources tested did not occur. However, in a subsequent study of 12 different isolates from filbert and 12 from walnut, the results with dextrose and sucrose were less consistent; both the walnut- and filbert-blight isolates showed almost as much variation between isolates within a group as between the two groups. Moreover, it was found that on extending the incubation period to 60 days, acid was produced by nearly all isolates from walnut and filbert on all sugars capable of being fermented. Since the agar slopes dried out extensively during prolonged incubation, the experiment was repeated, using liquid media. Sugars, alcohols, glucosides, and sodium salts of organic acids were added to the modified synthetic medium³ of Ayers, Rupp, and Johnson (1), as recommended in the Manual of Methods for Pure Culture Study of Bacteria, issued by the Society of American Bacteriologists (8). The basal medium was adjusted to pH 7 and was sterilized by autoclaving. Brom-cresol purple was added as an indicator, except in the media containing organic salts, where brom-thymol blue was employed. The indicator concentration in all cases was 0.001 per cent in the basal medium. All of the carbon sources were prepared in 5 per cent concentrations with distilled water and sterilized by filtration through a glass filter (Jena G3). One cc. of the solution was added aseptically to 4 cc. of the sterilized basal medium in small test tubes, giving a concentration of 1 per cent carbon source in the final medium. All media were incubated for 5 days at 28° C. to check their sterility before using. To test for the utilization of cellulose, strips of acid-washed filter paper were added to test tubes containing the basal medium, and these were sterilized in the autoclave. Utilization of starch was determined by streaking starch agar plates made with the basal medium and containing brom-cresol purple as an indicator. The iodine method was used to detect starch digestion. Inoculations of all liquid media were made in duplicate. One loop of a distilled-water suspension prepared by mixing two loops of growth from a 24-hour-old dextrose-nutrient-agar culture with 10 cc. of sterile water was used as the inoculum. Quantitative tests showed that this inoculum averaged several hundred thousand bacteria. Cultures were incubated at 28° C. and observed daily. The synthetic media permitted only relatively slow growth, from 3 to 5 days elapsing before any turbidity appeared. Tubes showing no turbidity within 60 days were considered negative for growth. Development of acidity was generally much slower than growth. The results of these carbohydrate-fermentation studies are given in table 1.

³ Basal medium for carbon sources:

NaNH ₄ HPO ₄ · 4H ₂ O	1.0	g.
KCl	1.0	g.
MgSO ₄ · 7H ₂ O	0.2	g.
Brom-cresol purple	0.01	g.
Distilled water	1000	cc.
Adjusted to pH 7.0 with NaOH solution		

TABLE 1.—*Growth of walnut- and filbert-blight pathogens in synthetic media containing various sources of carbon*

Carbon source	Days required to produce indicated changes						Filbert-blight pathogens					
	Walnut-blight pathogens			Days required to produce indicated changes			No. of isolate					
	5056	5080	5154	5171	5184	5092	5146	5151	5164	5170	5256	
Dextrose	+10 ^a	+14	+14	+14	+30	+14	+10	+14	+10	+10	+10	+10
Levulose	+21	+21	+21	+21	+21	+21	+21	+21	+21	+21	+21	+21
Sucrose	+31	+31	+31	+28	+28	+28	+28	+28	+28	+28	+28	+28
Lactose	+33	+14	+14	+33	+27	+16	+14	+14	+14	+14	+14	+14
Maltose	+27	+27	+32	+27	+14	+70	+27	+33	+14	+14	+14	+14
Raffinose	+42	+27	+42	+52	+42	+42	+27	+42	+27	+42	+42	+42
Arabinose	0 ^b	0	0	0	0	0	0	0	0	0	0	0
Rhamnose	0	0	0	0	0	0	0	0	0	0	0	0
Xylose	+22	+32	+32	+32	+32	+36	+32	+22	+32	+32	+32	+32
Mannitol	+17	+13	+13	+13	+13	+13	+13	+13	+13	+13	+13	+13
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	+32	+22	+22	+32	+22	+32	+22	+32	+32	+32	+32	+32
Salicin	0	0	0	0	0	0	0	0	0	0	0	0
Starch	+13 ^c	+13	+13	+13	++	++17	++13	++13	++	++	++	++
Inulin	0	0	0	0	0	0	0	0	0	0	0	0
Cellulose	0	0	0	0	0	0	0	0	0	0	0	0
Sodium acetate	0	0	0	0	0	0	0	0	0	0	0	0
Sodium benzoate	0	0	0	0	0	0	0	0	0	0	0	0
Sodium citrate	0	0	0	0	0	0	0	0	0	0	0	0
Sodium formate	-5 ^d	-5	-7	-5	-5	-5	-5	-5	-5	-5	-5	-5
Sodium lactate	0	0	0	0	0	0	0	0	0	0	0	0
Sodium malate	-13	-13	-13	-13	-13	-13	-13	-13	-13	-13	-13	-13
Sodium salicylate	-7	-7	-7	-7	-7	-7	-7	-7	-7	-7	-7	-7
Sodium succinate	0	0	0	0	0	0	0	0	0	0	0	0
Sodium tartrate	0	0	0	0	0	0	0	0	0	0	0	0

a + Indicates heavy growth and full acid color of brom-cresol purple; figure following (+) sign indicates number of days required to produce full acid color of indicator.

b (0) Indicates no growth within 60 days.

c (++) Indicates hydrolysis of starch and full acid color of brom-cresol purple; figure following indicates number of days required to produce full acid color of indicator.

a (-) Indicates heavy growth and full alkaline color of brom-thymol blue; figure following indicates number of days required to produce full alkaline color of indicator.

As shown in this table, the most rapid change occurred with 1 particular isolate from walnut, No. 5184, and 3 filbert-blight isolates, Nos. 5164, 5170, 5256, on starch, full acid color of brom-cresol purple being attained in 2 days. This was exceptional, since all the other isolates required 13 days to produce a corresponding change, which compares closely with the average time required to ferment dextrose. The most consistent rapid fermentation by all isolates occurred with sodium citrate, only 5 to 7 days being required for development of the full alkaline color of brom-thymol blue. All isolates completed the indicator change in 7 days with malate and succinate. Since the substrates in these 3 instances are relatively simple anions, a different fermentation mechanism is probably involved, which could explain the more rapid changes observed. With some of the sugars the isolates showed a great variation in rate of action; maltose, for example, was fermented to full acid color of brom-cresol purple in from 14 to 70 days by the isolates from filbert, and in from 14 to 32 days by the isolates from walnut. Levulose, on the other hand, was fermented at a uniform rate, the indicator being fully changed by all isolates in 21 days. This is, however, an unusually slow change, levulose being typically fermented as rapidly as dextrose by most other bacteria (22). Good agreement in duplicate cultures was obtained; in a few cases, they differed by 2 to 10 days in time required to completely change the indicator. The data in table 1 are averages for duplicate tubes. In decreasing order of average rate of utilization by all walnut- and filbert-blight isolates, the carbon sources can be listed as follows: citrate, malate, succinate, starch, lactate, mannitol, dextrose, lactose, levulose, maltose, glycerol, sucrose, xylose, and raffinose. The filbert-blight isolates averaged a more rapid fermentation of dextrose, sucrose, lactose, raffinose, mannitol, starch and citrate, while the average for the isolates from walnut was greater with maltose, xylose, and glycerol. With the other carbon sources there was no difference between the filbert- and walnut-blight isolates in rate of acid production. Arabinose, rhamnose, dulcitol, salicin, inulin, cellulose, acetate, benzoate, formate, salicylate, and tartrate did not permit growth of any isolate. If one isolate could grow with a given carbon source, all other isolates also could utilize it. Differences appeared only in the rate of utilization and these were not consistent, being fortuitously distributed among all isolates, and among most of the carbon sources. Through an oversight galactose was not included with the carbon sources for the study reported in table 1. However, in the preliminary studies, it was found to yield acid without gas and is, therefore, included in the technical description.

It would appear from the foregoing that differences in the carbon metabolism of the filbert-blight pathogen and *Phytomonas juglandis* are insignificant and that these two pathogens cannot be differentiated by variations in the carbon metabolism.

In his recent paper Dowson (9) reports that *Phytomonas juglandis*, contrary to our results, produces no acid from sucrose, xylose, and glycerol. The shorter incubation period, not over 4 weeks, used by Dowson, may account for this discrepancy.

Nitrogen Metabolism.—There are a number of nitrogen compounds that can support the growth of the filbert- and walnut-blight pathogens. Availability of various nitrogen sources was determined by using 0.1 per cent concentrations in a basal synthetic medium⁴ containing dextrose. The nitrogen sources were prepared in 5 per cent solution and added aseptically to autoclaved tubes of the basal medium. The sodium nitrite was sterilized by filtration; all other nitrogen sources were autoclaved at 15 pounds' pressure for 20 minutes. Inoculations were made as previously described and the cultures were incubated at 28° C. Comparisons were made on the basis of time required to produce heavy growth as indicated by a turbidity comparable to that shown by a 48-hour-old culture of *Escherichia coli* in standard nutrient broth. The results of these studies appear in table 2.

TABLE 2.—Growth of walnut- and filbert-blight pathogens in synthetic media containing various sources of nitrogen

Nitrogen source	Days required to produce heavy growth ^a									
	Walnut blight pathogens					Filbert blight pathogens				
	No. of isolate					No. of isolate				
	5056	5080	5154	5171	5184	5092	5146	5151	5164	5170
Alanine	10	10	14	14	10	14	10	10	10	10
Allantoin	12	12	12	12	12	12	12	12	12	12
Aspartic acid ...	10	10	10	10	10	10	10	10	10	10
Brucine	18	18	18	18	18	18	18	18	18	18
Glutamic acid ...	0	0	0	0	0	0	0	0	0	0
Hippuric acid ...	0	0	0	0	0	0	0	0	0	0
Leucine	14	14	10	10	10	10	10	14	10	10
NaNH ₄ HPO ₄	10	14	14	14	30	14	10	14	10	10
NaNO ₂	0	0	0	0	0	0	0	0	0	0
Peptone	2	2	2	2	2	2	2	2	2	2
Tyrosine	12	12	12	12	12	12	12	12	12	12
Uric acid	12	12	12	12	12	12	12	12	12	12

^a As indicated by a turbidity comparable to that shown by a 48-hour culture of *Escherichia coli* in standard nutrient broth. All cultures reported (0) showed no growth within 60 days.

The data presented in table 2 show that greater uniformity exists in the ability of the various isolates to utilize the same as well as different nitrogen sources than was the case with carbohydrate utilization. Peptone gave the most rapid growth, all walnut- and filbert-blight isolates producing heavy turbidity in 2 days. This was to be expected from general experience with the growth of these organisms on standard laboratory media containing dextrose and peptone. Rapid growth in the presence of peptone is probably due to accompanying neutralites. Slowest growth was obtained with brucine, 18 days being required by all isolates to produce turbidity compar-

⁴ Basal medium for nitrogen sources:

K ₂ HPO ₄	1.0 gram
NaCl	0.2 "
MgSO ₄ · 7H ₂ O	0.2 "
Dextrose	10.0 "

Adjusted to pH 7.0 with NaOH solution.

able to a 2-day culture in peptone; this is excepting the 30-day period required by one walnut isolate with sodium ammonium phosphate. All other cultures attained heavy growth in from 10 to 14 days. Listed in order according to average effect on rate of growth of all isolates, the nitrogen sources utilized are peptone, aspartic acid, alanine, leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid and brucine. Glutamic acid, hippuric acid, and sodium nitrite did not support the growth of any isolate.

From these results it is evident that the filbert-blight pathogen cannot be distinguished from *Phytomonas juglandis* on the basis of nitrogen source utilization.

Temperature Relations.—Studies of the temperature relations of one isolate of the filber-blight pathogen from filbert and one isolate of *Phytomonas juglandis* from walnut indicate that these two organisms have approximately similar temperature relations. The optimum temperature for the growth of both pathogens in culture was between 28° and 32° C., the maximum about 37° C. and the minimum between 4° and 7° C.

Thermal Death Point.—With a 10-minute exposure in Difco beef-extract-dextrose broth, the thermal death point of one filbert-blight isolate and one isolate of *Phytomonas juglandis* from walnut was the same, namely, between 53° and 55° C.

Agglutination Relationships.—Results of preliminary agglutination studies seem to indicate that the filbert-blight pathogen may be serologically different from *Phytomonas juglandis*. In order to observe the serological relationships of the walnut and filbert isolates, agglutinating sera were produced by using five walnut isolates and four filbert isolates. The growth from 48-hour-old dextrose-nutrient-agar-slope cultures was suspended in 10 cc. of physiological salt solution. One cc. of the suspension from each culture was injected intravenously into full grown rabbits. Gradually increasing amounts of the various antigens were injected until some of the animals were given 5 cc. There appeared to be considerable variation in the toxicity of the antigens of both the filbert and walnut isolates. In some of the rabbits the reactions were so severe that several of them succumbed. Since the injections were given at 3-day intervals, it would not ordinarily be expected that the animals had become sensitized to a foreign protein. Where it occurred, death took place from 2 hours to 18 hours after the antigen had been injected. Later, antigens from these apparently toxic cultures, that had been heated to 56° C. for 1 hour were used. No severe reactions occurred, even when large amounts of the heated antigens were used. The antigenic response of the various rabbits was variable. In some, a titer of 1-10,000 could be easily obtained; with others the titer was very much lower. With one or two isolates, no agglutinin was obtained. Whether the cultures lacked antigenic qualities, or the rabbit would not respond to the organism, has not yet been studied.

Cross-agglutination tests were made to observe if any specific agglutina-

tion of isolates could be obtained. With the walnut isolates, there was virtually no evidence of specific isolate agglutinin, as all the walnut isolates were agglutinated regardless of what walnut serum or isolate was used. In the case of the filbert isolates there was considerable evidence to indicate that there were some differences in the various antigenic agents. One isolate was agglutinated by its own specific serum only. All others cross-agglutinated in the lower dilution of the serum. In some agglutinin-absorption tests carried out on the various organisms, there was no evidence to indicate that, after absorption, the sera contained any agglutinating antibody for any of the isolates. This was observed with both walnut and filbert isolates. Using serum produced by the walnut isolates, agglutination tests were made with the filbert isolates. There was no demonstrable agglutination. Likewise, when sera produced by the filbert isolates were tested with the walnut isolates, no agglutination was observed. In order to check the accuracy of the agglutination tests in separating the walnut and filbert isolates, tests were made on 10 unknown cultures without any knowledge of their origin. Two of the cultures agglutinated with walnut serum, 6 of them agglutinated with the filbert serum, while the other two cultures did not agglutinate with either sera. These latter two cultures proved upon identification to be cultures of *Phytophthora phaseoli*, the etiological agent of bean blight. All 8 of the filbert and walnut isolates were correctly identified.

More recently, further agglutination studies were made on 32 recent isolates from walnut and 21 from filbert. The isolates came from widely separated areas and there were no duplicate isolates from the same orchard. Without any knowledge of whether they were filbert or walnut isolates, each one was correctly identified by agglutination tests. Seven additional isolates from filberts did not agglutinate with sera produced by either the walnut or filbert organism. There was no cultural evidence to indicate that these particular isolates had dissociated. Their pathogenicity is now being checked to determine if they are still virulent.

The results of these preliminary cross agglutination studies seem to suggest, therefore, that the filbert-blight pathogen may be serologically different from *Phytophthora juglandis*. Further study is needed, however, to definitely determine serological relationships.

Pathological Characteristics

In nature the filbert-blight pathogen is actively parasitic on buds, leaves, stems of shoots of current growth, and on branches and trunks 1 to 4 years of age and occasionally older, but only very mildly pathogenic on the nuts of *Corylus avellana* and *C. maxima*. The walnut-blight pathogen, on the other hand, is aggressively pathogenic on the nuts, buds, leaves, and shoots of current growth of *Juglans regia*, but does not attack branches 1 year of age or older.

Cross-infection Studies.—The striking similarity in the morphological, cultural, and biochemical characteristics of the walnut- and filbert-blight

organisms has given rise to the question of pathological similarities or differences between these two pathogens. To determine pathological relationships, numerous cross-infection studies on *Corylus avellana* and *J. regia* have been performed over a period of 7 years both in the field under natural conditions and upon potted plants in the greenhouse under artificial conditions. In the following pages there is discussion of methods used and a summary of the results obtained from the cross-inoculation studies made.

On Leaves.—Numerous lesions were produced on young leaves of potted walnut trees in the greenhouse by atomizer inoculations with each of 5 isolates from filbert. The trees were given a 24-hour preinoculation moist treatment in a damp chamber in a saturated atmosphere and were kept at 100 per cent humidity for 48 hours thereafter. The lesions that developed were microscopically indistinguishable from those produced in parallel inoculations with each of 5 isolates from walnut. Likewise, many similar lesions were produced on young leaves of potted filbert trees by atomizer inoculations with each of 4 isolates from walnut and with each of 5 isolates from filbert. The trees were given a preinoculation moist treatment of from 24 to 48 hours and were kept at 100 per cent humidity for 48 hours afterwards. The results of these cross-inoculation studies with leaves would seem to suggest that the walnut- and filbert-blight pathogens are very closely related, if not identical, pathologically. However, in the light of recent studies by Johnson (12), the results of the aforementioned cross-infection studies appear to be of relatively less significance in respect to pathogenicity than those on other portions of the host to which reference will be made later. Johnson found that the leaves of a number of species of plants normally immune from infection by *Phytophthora angularata* and *P. tabaci* and certain other bacterial organisms become highly susceptible to infection by these organisms when the tissues are water-soaked. The methods of moist treatment in a damp chamber of saturated atmosphere before and after inoculation, as employed by the writers, resulted in a water-soaked condition of the foliage thereby permitting of positive and very similar infections by both pathogens in both hosts, even though on stems the differences in pathogenicity were clear cut.

On the Fruits.—Lesions were produced on half grown fruits of Persian walnut, grown under field conditions, by atomizer inoculations with each of two isolates from filbert. The lesions were very small, however, measuring from 1 to 2 mm. in diameter and not more than 2 mm. deep (Fig. 2, A). The lesions that developed from parallel atomizer inoculations made with each of two isolates from walnut were much larger, measuring from 4 to 12 mm. in diameter and extending down to the shell in many cases. (Fig. 2, B.) Replications of this experiment gave similar results.

Lesions also were produced on young fruits in the early post-bloom stage on potted filbert trees in the greenhouse by atomizer inoculations with a pure water suspension of one isolate from walnut. The inoculated trees were kept at 100 per cent humidity for 28 hours after inoculation. The lesions that developed were, however, very small and superficial, never penetrating more

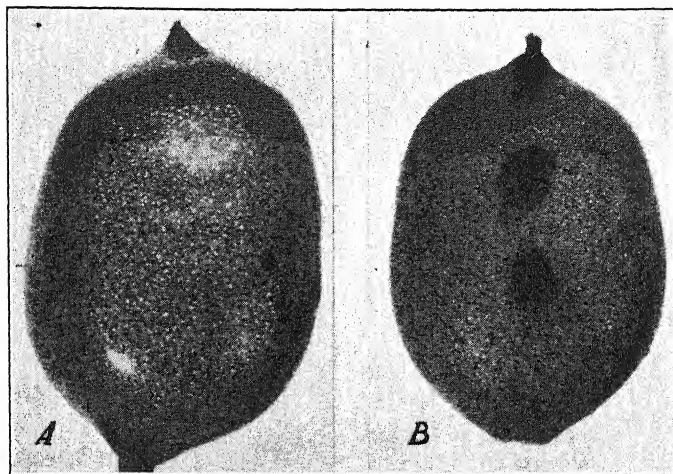


FIG. 2. Lesions on field-grown walnuts inoculated when about half grown by atomizing with pure water suspensions of isolates: A, from filbert; B, from walnut. Note variation in size of lesions, particularly the small size of lesions in A. About $\times 1$.

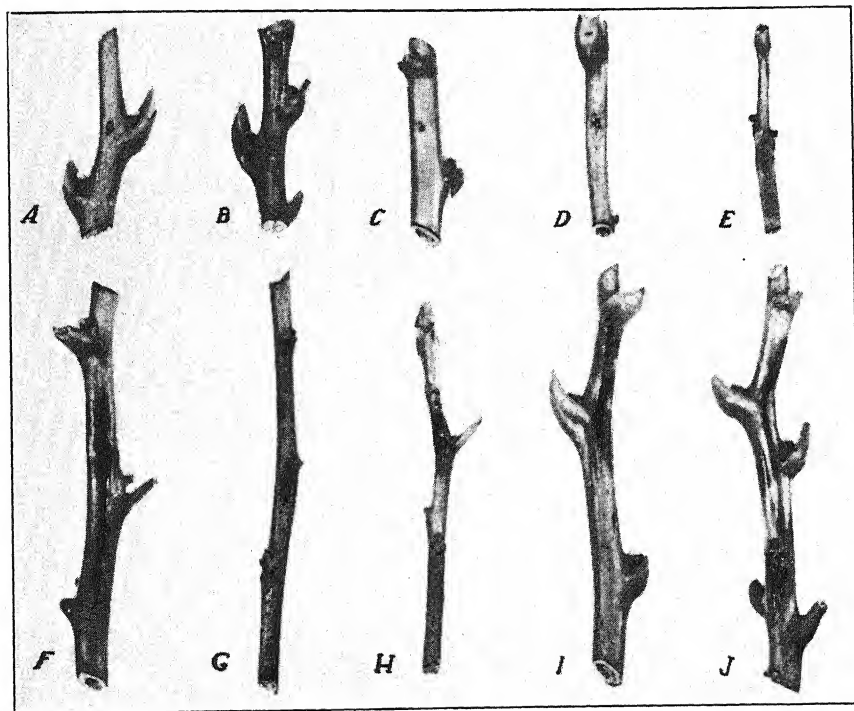


FIG. 3. Field-grown walnut stems of current growth inoculated by puncturing the tissues: A, sterile needle; B-E, 4 different isolates from filbert; F-J, 5 different isolates from walnut. Note absence of lesions about inoculations with the filbert isolates and the presence of comparatively large, well-defined lesions about inoculations with isolates from walnut. About $\times \frac{1}{2}$.

than 1 mm. into the shell. In this respect they did not differ from the lesions produced by parallel atomizer inoculations on young filbert fruits by an isolate from filbert. In nature, as stated previously, the filbert-blight pathogen is not actively parasitic on fruits of its natural host.

Although the isolates from filbert produced very small lesions on walnut fruits, the isolates from walnut were always consistently more virulent on their own host than all filbert isolates.

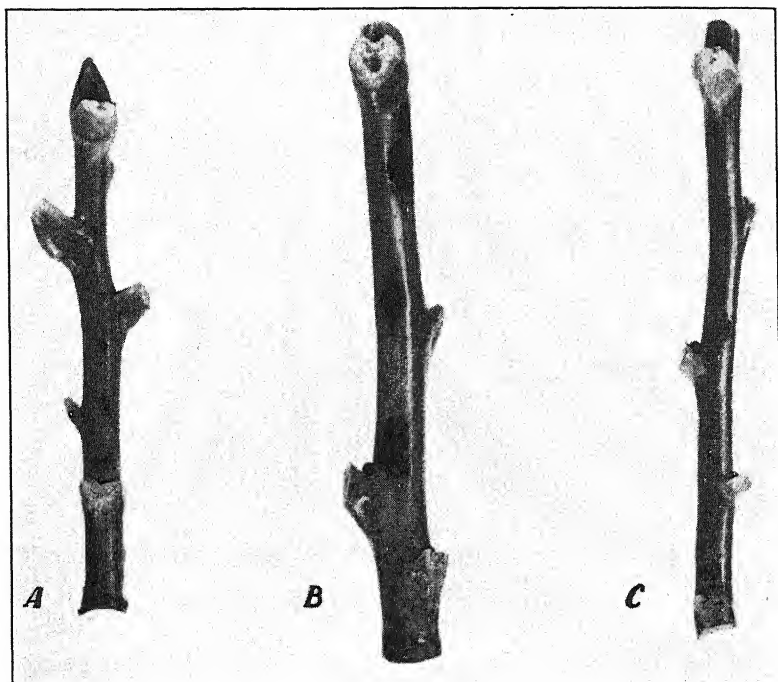


FIG. 4. Walnut stems of current growth on potted trees in greenhouse, inoculated by puncturing the tissues: A, sterile needle; B, isolate from walnut; C, isolate from filbert. About $\times 1$.

On Stems of Young Shoots of Current Growth.—No lesions, or only very small dead areas not exceeding 2 mm. in width, developed about puncture inoculations made in stems of young, field-grown walnut shoots of current growth with each of 7 different isolates from filbert, while large, well-defined cankers up to 50 mm. in their maximal diameter dimension developed about parallel puncture inoculations made in young shoots of walnut with each of 8 isolates from walnut. (Fig. 3.) Similarly, puncture inoculations made in stems of young walnut shoots of current growth on potted trees in the greenhouse with 1 isolate from filbert were negative; whereas large, well-defined lesions up to 12 mm. in their maximal diameter dimensions developed about parallel puncture inoculations made in stems of young walnut shoots with an isolate from walnut. (Fig. 4.)

No lesions, or only very small dead areas not exceeding 1 mm. in width,

developed about puncture inoculations made in stems of young filbert shoots of current growth on potted trees in the greenhouse with a typical isolate from walnut; whereas large, well-defined lesions up to 10 mm. in their greatest dimension developed about parallel puncture inoculations made in stems of young filbert shoots with a filbert-blight isolate from filbert.

On Older Branches.—No lesions, or only very small dead areas not exceeding 2 mm. in width, developed about puncture inoculations made in one-year-old twigs of filbert (*C. avellana*) with each of 5 different isolates from walnut; while large, well-defined cankers, many of which girdled the twigs and caused a dieback of the inoculated branches developed about parallel puncture inoculations made in one-year-old filbert twigs with each of 5 different isolates from filbert. (Fig. 5.) This experiment was repeated with similar results.

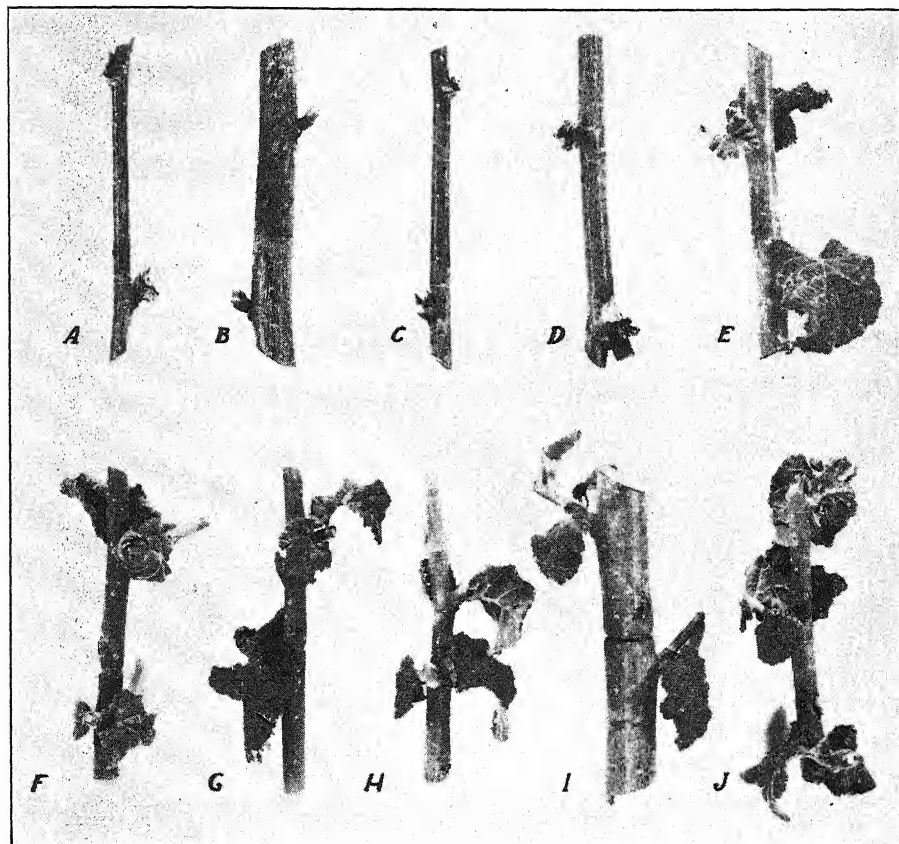


FIG. 5. Field-grown filbert branches, one year of age, inoculated by puncturing the tissues: A-D, 4 different isolates from filbert; E, sterile needle; F-J, 5 different isolates from walnut. Note absence of lesions about puncture inoculations with all isolates from walnut as denoted by healthy shoots above and below point of inoculation. Inoculations with filbert isolates in all cases have girdled the twigs and caused a dying back of the inoculated branches as indicated by presence of dead buds above and below point of inoculation.

Discussion of Cross-inoculations

It is apparent from the foregoing sections that there are distinct pathological differences between these 2 pathogens. No matter what isolates were compared in parallel inoculation tests under similar conditions, every isolate from walnut used was aggressively more parasitic, producing definitely larger lesions on walnut (except in the case of young foliage, which, in the light of Johnson's recent studies, are of but little or no significance with respect to relative pathogenicity) than any isolate from filbert used at the same time in comparison with it.

The difference in pathogenic behavior of these 2 organisms was especially evident in their pathological effects on the twigs and branches of each host. As stated above all isolates from walnut were negative or practically so on filbert branches, while all filbert isolates were negative on walnut stems of current growth. This difference in pathological behavior has been so consistent in the numerous cross-inoculation studies carried on, in both the present and earlier investigations, as to preclude any possibility of accidental differences due to the chance selection of a few isolates that behaved in the manner indicated.

General Discussion and Conclusions

The foregoing studies definitely show that although the filbert blight pathogen is very closely related to, if not identical, morphologically, culturally, and biochemically with *Phytophthora juglandis* there are distinct differences in pathogenic behavior. To properly indicate this distinction by appropriate nomenclature is a difficult problem. Consultation with a number of able bacteriologists and others interested in taxonomy disclosed lack of agreement as to whether or not it is sound policy to set up as a new species a bacterial plant pathogen that can be distinguished from one already described solely on the basis of a single consistent difference, namely, a difference in pathogenic effects on certain host species. There apparently is no generally established or widely recognized policy bearing on this question, and the writers are not themselves in complete agreement. The conservative course to follow would undoubtedly be to designate the filbert-blight pathogen as a variety of the walnut-blight organism to indicate the exceedingly close relationship suggested by the similarities between the two. However, prevailing practice in the definition of species within a group of closely related bacteria seems to be largely a matter of utility. If, then, an organism be specific and consistent in its pathogenicity, even though this provide practically the only means for differentiating it from some other organism likewise specific and consistent in pathogenicity, it would seem fitting to regard the two organisms as distinct and for the convenience of those working with them they might as well be designated as different species. Such a designation, even if not conforming strictly to a "natural" classification, would have advantages, since it is easier and simpler to recognize *P. juglandis* and *P. corylina* n. sp. than to recognize *P. juglandis* var. *juglandis* and *P. juglandis* var. *corylina*.

On the basis, therefore, of demonstrated pathological differences, and with supporting evidence from cross-agglutination studies, the filbert-blight pathogen is designated a new species, *Phytomonas corylina*.⁵

Technical Description

The organism is a short rod $1.1\ \mu$ to $3.8\ \mu \times 0.5\ \mu$ to $0.7\ \mu$, arranged singly or in pairs with rounded ends; motile by 1 polar flagellum and capsulate but without endospores; Gram-negative and not acid-fast; stains readily with gentian violet and carbol fuchsin, but only very lightly with methylene blue; growth at room temperature (about 22°C.) on nutrient dextrose-agar streaks is abundant, filiform, convex, glistening, smooth, opaque, pale lemon-yellow (coppery-yellow: No. 1 (23)), viscid and odorless; medium unchanged; dextrose-agar surface colonies are circular, over 1 mm. in diameter, round, glistening, pale lemon-yellow (coppery-yellow: No. 1 (23)), convex, entire margins and finely granular within; growth in nutrient broth is abundant and a ring forms at the surface after 2 to 5 days; on gelatin stabs growth is best at the top, liquefaction is stratiform, beginning in 1 day but not complete until from 4 to 6 weeks, the medium is unchanged except for a slight turbidity; aerobic; nitrates are not reduced to nitrites; gas is not formed; no indole or hydrogen sulphide produced; milk (casein) slowly digested and rennin is produced; selenium dioxide and litmus in litmus milk are reduced; starch is hydrolyzed; ammonia is produced in nutrient broth containing peptone. At 28°C. acid, but no gas, is produced from dextrose, levulose, galactose, lactose, sucrose, maltose, xylose, raffinose, mannitol, glycerol, and starch; no acid or gas produced from arabinose, rhamnose, dulcitol, salicin, inulin and cellulose; alkali is produced from citrate, lactate, malate, and succinate. At 28°C. acetate, benzoate, formate, salicylate, and tartrate are not fermented. Nitrogen sources utilized at 28°C. in the order of their availability are peptone, aspartic acid, alanine, leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid, and brucine. Glutamic acid, hippuric acid, and sodium nitrite are not utilized. Optimum temperature for growth is 28°C. to 32°C. ; maximum 37°C. , minimum 5° to 7°C. ; thermal death point 53° to 55°C. ; the pH range for growth pH 5.2 to 10.5, optimum reaction for growth pH 6 to 8.

Actively pathogenic to buds, leaves, stems of shoots of current growth, branches and trunks mostly from 1 to 4 years of age of cultivated filberts, *Corylus avellana* and *C. maxima*, and weakly pathogenic to nuts of the same hosts in the region from the Cascade mountains westward in Oregon and Washington.

⁵ The recent note of Elliott (10) does not appear to the authors to justify rejection at this time of the name *Phytomonas* for this genus of bacterial plant pathogens. Although Dowson (9) contends that *Phytomonas* is suppressed it is retained in the new (1939) edition of Bergey's Manual (4, 7). When adequate taxonomic revision is possible it would seem that *Xanthomonas*, proposed by Dowson (9), could properly replace *Phytomonas* for the group of related plant pathogenic bacteria, which belong in neither *Pseudomonas* nor *Erwinia*.

SUMMARY

The morphological, cultural, biochemical, and pathological characteristics of a number of isolates of the filbert blight pathogen, a yellow, capsulate rod with a single polar flagellum are described and compared with those of a number of isolates of *Phytomonas juglandis* (Pierce) Bergey *et al.*, the cause of walnut bacteriosis.

Essentially no differences in morphology, staining reactions, cultural characters, or biochemical characteristics were found between the two pathogens.

While carbon metabolism studies revealed no differences between the pathogens in regard to carbon sources utilized, differences did appear in the rate of utilization of certain carbon sources by different isolates. These differences, however, were fortuitously distributed between all isolates of both pathogens and were of no differential value.

Both pathogens utilized the same nitrogen sources. Variations in rate of utilization of nitrogen sources by different isolates were found but these were less pronounced than variations in rate of utilization of carbon sources.

Results of preliminary cross-agglutination studies suggest that the filbert-blight pathogen may be serologically separated from *Phytomonas juglandis*.

Distinct differences in pathogenic behavior between *Phytomonas juglandis* and the filbert-blight pathogen were demonstrated. In cross-inoculation studies, carried on under field conditions, all isolates from walnut were negative or practically so on filbert branches, whereas all filbert isolates were negative on walnut stems of current growth.

On the basis of pathological and serological differences, it is proposed to designate the filbert-blight organism as a new species, *Phytomonas corylina*.

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RESISTANCE OF CERTAIN POTATO VARIETIES AND SEEDLING PROGENIES TO LATE BLIGHT IN THE TUBERS

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INTRODUCTION

Late blight, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, is one of the limiting factors in potato production, and its control is a very important problem. The average loss from late blight in the United States for the 10-year period 1925-1934 was estimated at 3.1 per cent of the total crop (6). The disease is present nearly every year in the New England States, and epidemics are not infrequent along the entire Atlantic Seaboard from Maine to southern Florida. In 1938 Vermont and New York each reported a loss of 35 per cent of the crop. Late-blight epidemics occur also in Pennsylvania and Ohio, and occasionally the ravages extended into Wisconsin and Minnesota (1). It also may be destructive in California and the lower Rio Grande Valley, as well as in other potato-growing sections.

It does more damage in Maine than any other potato disease. According to the estimates published in the annual supplements of the Plant Disease Reporter, U. S. Department of Agriculture, on losses from diseases, the average annual loss from potato late blight in Maine for the 14-year period,

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1924 to 1938, was 3,790,786 bushels. This amount is 8.9 per cent of the average annual yield recorded in the same supplements for the State during this period. These losses have occurred in spite of large yearly expenditures for fungicides, labor, and equipment for combating the disease.

Studies conducted in Maine, pertaining to the problem of breeding for resistance to late blight of the potato, have been published by Stevenson *et al.* (4, 5). The present paper is a result of the continuation of this work, with the emphasis on resistance in the tubers and its relation to resistance in the foliage.

COMPARISON OF VARIETIES AND SEEDLINGS

Materials

These studies were conducted mainly with the seedling progenies of different crosses and of one selfing, as well as with their parents. The parents were known to vary from extreme susceptibility to fair resistance, as will be disclosed in the discussion of various tests. Other miscellaneous varieties and seedlings were studied also and their behavior will be discussed.

Methods

These studies were conducted at Presque Isle, Maine. The seedlings and varieties tested for resistance to the disease were grown in duplicated 10-hill lots in a wet field that was suitable for the development of tuber rot caused by the late-blight fungus when the disease was prevalent. The Green Mountain variety was used as the susceptible check and was planted in alternate rows with the other varieties in the test. This variety was included also as a source of inoculum for spread of the disease. The parents of the various crosses were included for comparison and were distributed throughout the plot.

A late-blight epidemic was artificially induced in the test plots in 1937 by spraying the plants several times at blossoming time with a suspension of zoospores washed from the infected leaves of a susceptible variety. The season of 1938 was exceptionally favorable for the disease and natural infection was adequate.

The tuber inoculations in 1937 and the previous years were made in the laboratory. The tubers were dug carefully to avoid bruises before being subjected to late-blight infection. They were inoculated by spraying with a water suspension of zoospores and were placed in moist chambers for observation. Each variety was tested 2 or 3 times before its reaction to the fungus was finally classified as to resistance. Records were obtained regarding the number of tubers that developed decay and also as to the part of the tuber infected.

The resistance to tuber decay in 1938 was judged by the amount of natural field infection. Rot caused by the late-blight fungus is very prevalent during epidemics, provided the potatoes are grown in Washburn loam and the plants are not sprayed with a fungicide. The different lots of potatoes were dug by hand and the tubers were examined individually.

The reaction of the foliage of the potato varieties studied was determined by estimating the amount of the foliage killed by the fungus under field conditions. Four different degrees of infection were used for classifying the reaction of the foliage to the disease, as follows:

- 0—Foliage very resistant to late blight and almost free from infection.
- 1—Foliage resistant, but with some infected spots, especially on lower leaves.
- 2—Foliage resistant, but more susceptible than in Class 1, with spots occurring on all leaves to the extent of infecting at least one leaflet per leaf.
- 3—Foliage very susceptible, but with some green leaves and stalks.
- 4—Foliage very susceptible; all shoots, including stalks, killed by late blight.

Results

The results of the data secured in these experiments will be discussed for the years 1937 and 1938, separately.

The results of the 1937 laboratory tests as to degree of resistance in each progeny are given in table 1, together with comparisons in the parents and

TABLE 1.—*Resistance of controls, parents, and progenies to tuber infection by late blight in 1937, as studied in the laboratory*

Variety or cross	Check and parent lots of tubers tested	Seedlings tested	Percentage of tested lots showing		
			Fast growth of fungus	Infection but slow growth of fungus	No infection
Green Mountain	20	100.0	0	0
Earlaine	8	100.0	0	0
Ekishirazu	10	0	10.0	90.0
Paisley No. 2	4	0	0	100.0
President	4	0	0	100.0
Paisley No. 2 × Earlaine	96	45.6	11.5	42.9
Paisley No. 2 × Ekishirazu	51	29.5	31.4	39.1

two check varieties. The tubers in the varieties Earlaine and Green Mountain were very susceptible and decayed rapidly when inoculated. In contrast, the tubers of Paisley No. 2 and President (No Blight) were resistant to infection and did not decay in these tests. The variety Ekishirazu also was resistant but became infected in some cases, followed by a slow decay.

A wide variation was found in the progeny of the cross Paisley No. 2 × Earlaine. Forty-two and nine-tenths per cent of the seedlings showed no tuber decay. Eleven and five-tenths per cent became infected but the fungus made slow growth, and 45.6 per cent were in the same class as Green Mountain; that is, they were easily infected and decayed rapidly when inoculated. The progeny of Paisley No. 2 × Ekishirazu showed very little more resistance than did that of Paisley No. 2 × Earlaine. This is somewhat surprising, since the tubers of Ekishirazu are much more resistant than those of Earlaine (Table 1).

The tests in 1938 were conducted as in 1937, with the exception, as noted previously, that the tuber decay was the result of natural infection in the field instead of being induced in the laboratory. The data for the reaction of the different varieties and progenies to tuber infection by late blight in 1938 are given in table 2.

TABLE 2.—*Resistance of checks, parents, and progenies to tuber infection by late blight in the field in 1938*

Variety or cross	Check and parent lots of tubers tested	Seedlings tested	Percentage of tested lots showing		
			Fast growth of fungus	Infection but slow growth of fungus	No infection
Green Mountain	187	100.0	0	0
Russet Rural	4	100.0	0	0
Earlaine	10	100.0	0	0
Katahdin	10	100.0	0	0
Hindenburg	10	50.0	0	50.0
Richter's Jubel	4	50.0	0	50.0
336-144	10	0	0	100.0
336-18	10	0	0	100.0
President	12	0	0	100.0
Paisley No. 2	6	0	0	100.0
Russet Rural × S 44537	6	100.0	0	0
Katahdin Selfed	27	78.0	0	22.0
Paisley No. 2 × Earlaine	104	76.1	0	23.9
Richter's Jubel × S 44537	20	40.0	30	30.0
Hindenburg × Katahdin	22	41.1	27.2	31.7
President × Earlaine	94	23.6	0	76.4
(336-144) × (336-18)	306	19.8	0	80.2

The varieties Russet Rural, Earlaine, and Katahdin, used as parents for the crosses, and the Green Mountain controls were very susceptible. The foreign varieties Hindenburg and Richter's Jubel were less readily infected than were the highly susceptible varieties used in these tests. The tubers of the vine-resistant varieties (336-144), (336-18), President and Paisley No. 2 were not infected.

Six seedlings of the cross Russet Rural × S 44537 were very susceptible.

The reactions of the seedlings obtained by selfing Katahdin are interesting. Twenty-two per cent were resistant to tuber decay. This shows that the phenotypically susceptible variety Katahdin carries a factor for resistance to tuber rot. The progeny of the cross between a resistant and a susceptible variety Paisley No. 2 × Earlaine showed about the same resistance as the progeny of the Katahdin selfed. Twenty-three and nine-tenths per cent of the seedlings of this cross were free from tuber rot. This is in contrast with the 42.9 per cent that escaped infection in the same cross in the 1937 tests. The difference here cannot be accounted for at present.

The seedlings from Richter's Jubel × S 44537 and Hindenburg × Katahdin were selected previously because of their resistance to common scab caused by *Actinomyces scabies*. It is interesting to note that approxi-

mately one-third of them were apparently resistant to tuber rot initiated by the late-blight fungus.

A comparison of the data for the two crosses President \times Earlane and (336-144) \times (336-18) shows them to be quite similar in their reaction to tuber rot, 76.4 per cent of the first and 80.2 per cent of the latter having escaped infection. It is apparent that Earlane, like Katahdin, although phenotypically susceptible, is carrying a factor or factors for resistance. Resistant tubers from the cross (336-144) \times (336-18) in comparison with the Green Mountain checks are shown in figure 1 (Table 2.)



FIG. 1. Resistance of tubers to late blight. Sound tubers are of two seedling varieties selected from the (336-144) \times (336-18) cross; decayed tubers are Green Mountain controls. Both lots were surface inoculated with spores of *Phytophthora infestans* in the same moist chamber.

Correlation of Tuber and Vine Resistance. The question arises as to whether or not resistance to tuber rot is correlated with vine resistance. The percentages of the progenies classified for vine resistance, together with the percentages of these classes that showed tuber resistance, are found in table 3.

The data indicate that resistance in tubers is associated with vine resistance in the progenies of Katahdin selfed, Hindenburg \times Katahdin (336-144) \times (336-18), and President \times Earlane. Eighty-six and three-tenths per cent and 94.4 per cent, respectively, of the crosses (336-144) \times (336-18) and President \times Earlane that were found in the 0 Class for vine re-

TABLE 3.—Checks, parents, and progenies with relationship shown between percentage of resistant tubers^a and the degree of foliage infection^b

Variety or cross	Check and parent lots of tubers tested	Seedlings tested	Percentage of progenies, in classes formed on basis of foliage infection, that have resistant tubers ^a						Percentage of progenies that have foliage infection in Classes 0, 1 and 2
			Class 0	Class 1	Class 2	Class 3	Class 4	All 5 Classes	
			Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Green Mountain	207	— ^d	—	—	0.0	0.0	0.0	0.0
Russet Rural	4	—	—	—	—	0.0	0.0	0.0
Earlaine	18	—	—	—	0.0	0.0	0.0	0.0
Katahdin	10	—	—	—	—	0.0	0.0	0.0
Hindenburg	10	—	50.0	—	—	—	50.0	100.0
Richter's Jubel	4	—	50.0	—	—	—	50.0	100.0
Ektishraza	10	90.0	—	—	—	—	90.0	100.0
.....	10.0 ^e	—	—	—	—	10.0	—
336-144	10	—	100.0	100.0	—	—	100.0	100.0
336-18	10	—	100.0	—	—	—	100.0	100.0
President	16	—	100.0	100.0	—	—	100.0	100.0
Paisley No. 2	10	—	100.0	100.0	—	—	100.0	100.0
Russet Rural	6	—	—	—	—	0.0	0.0	0.0
× S 44537	27	100.0	50.0	0.0	0.0	23.5	22.2	33.3
Katahdin Selfed
Paisley No. 2	7.3	51.1	26.3	18.5	33.3	33.4	64.7
× Earlaine	200	4.2 ^e	30.0 ^e	55.1 ^e	3.7 ^e	—	5.7 ^e	—
Paisley No. 2	70.0	45.4	7.6	0.0	—	39.1	86.3
× Ektishraza	51	30.0 ^e	45.4 ^e	20.0 ^e	25.0 ^e	—	31.4 ^e	—
Richter's Jubel	66.7	42.9	42.9	0.0	0.0	30.0	85.0
× S 44537	20	42.9 ^e	28.6 ^e	30.0 ^e

TABLE 3.—(Continued)

Variety or cross	Check and parent lots of tubers tested	Seedlings tested	Percentage of progenies, in classes formed on basis of foliage infection, that have resistant tubers ^a							Percentage of progenies that have foliage infection in Classes 0, 1 and 2
			Percentage of progenies, in classes formed on basis of foliage infection, that have resistant tubers ^a							
			Class 0	Class 1	Class 2	Class 3	Class 4	All 5 Classes		
			Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Hindenburch	22	100.0	37.5	13.6	0.0	—	31.7	86.4	
× Katahdin	62.5 ^e	13.6 ^e	27.2 ^e	
President	94	94.4	73.1	65.7	100.0	33.3	76.4	84.0	
× Earlane	306	86.3	87.3	77.0	68.4	54.2	80.2	85.9	
(336-144)									
× (336-18)									

^a Percentage of resistant tubers is taken from tables 1 and 2.

^b 0 = Foliage very resistant to late blight and almost free from infection.

^c 1 = Foliage resistant, but with some infected spots, especially on lower leaves.

^d 2 = Foliage resistant, but more susceptible than in Class 1, with spots occurring on all leaves to the extent of infecting at least one leaflet per leaf.

^e 3 = Foliage very susceptible, but with some green leaves and stalks.

^f 4 = Foliage very susceptible; all shoots, including stalks, killed by late blight.

^g Arranged about as in tables 1 and 2.

^h A dash (—) indicates that there were no progenies with foliage in the class indicated, while 0.0 means that there were progenies with foliage in the class indicated but no progenies with resistant tubers.

ⁱ Infected but resistant to rapid decay.

sistance were also free from tuber rot. In the cross Paisley \times Earlane, however, only 7.3 per cent of the 0 Class for vines had apparently resistant tubers.

The cross Richter's Jubel \times S 44537 also had a considerable percentage in Class 0 for foliage resistance, but with tubers susceptible to blight decay (Table 3.)

Tuber resistance usually was present to a somewhat less degree in the progenies with foliage in the less resistant Class 1. The varieties Paisley No. 2, President, and seedlings (336-18) and (336-144), all with foliage in Class 1, possessed tubers that failed to decay in these tests. The progeny of President \times Earlane and (336-144) \times (336-18) with foliage reactions in this class continued to show a high percentage with resistant tubers, namely, 73.1 and 87.3 per cent, respectively. The progenies in the other crosses and varieties with a Class 1 reaction to foliage infection were less resistant to tuber infection, the number with resistant tubers varying from 37.5 to 51.1 per cent.

Class 2 possesses a considerable amount of foliage resistance to late-blight infection, although the disease does cause severe spotting of the leaves. It may be noted that tuber resistance persisted in this class to a rather large extent in the crosses (336-144) \times (336-18) and President \times Earlane. These crosses had 77.0 and 65.7 per cent, respectively, of the seedling progenies in Class 2 with resistant tubers. It is also apparent that the other crosses having progenies with foliage in Class 2 were less resistant to tuber decay than those referred to just previously.

That tuber resistance to late-blight infection is not always associated with resistance in the foliage is apparent in the reaction of the tubers listed under the susceptible foliage Classes 3 and 4, in table 3. Seedlings with susceptible foliage and resistant tubers were found in the following crosses: (336-144) \times (336-18), President \times Earlane, Paisley No. 2 \times Earlane, and Katahdin selfed.

Approximately 63 per cent of the progeny of Katahdin selfed were very susceptible to foliage infection and were killed by the disease. Twenty-three and five-tenths per cent of these seedlings, however, had tubers that were resistant to rot. Although the results of these experiments are not conclusive, it is evident that tuber resistance is a heritable character and that it is not conditioned by the same genetic factors as vine resistance. The high correlations between the two in some crosses might indicate genetic linkage; but this may be a pseudo-relationship, since, in crosses with a high percentage of seedlings with very resistant vines, the tubers would have greater chances of escape in the field and the resulting correlation would be too high. The interesting fact from the breeder's standpoint is that vine resistance and resistance to tuber rot occur together in relatively high percentages of certain progenies and it should not be difficult to select varieties with a combination of both characters.

NATURE OF RESISTANCE

Resistance in the Foliage

The foliage of some varieties studied in the field escaped infection because of certain growth habits. Varieties with erect or sparse foliage that is easily aerated are affected less by blight infection than those with a dense and heavy foliage. The foliage of the latter remains wet for long periods of time following rains or heavy dews, a condition that favors rapid spread of blight. In this study, lack of blight infection, when due to escape because of growth habit, has not been considered true resistance.

The fungus grows rapidly and sporulates abundantly in the foliage of Green Mountain, Irish Cobbler, Katahdin, and many other commercial varieties and the plants are soon destroyed. Apparently the fungus gains entrance readily and progresses rapidly within the foliage. In some of the other varieties studied, the fungus grows well, provided infection occurs. However, infection spots are relatively few in number and the plants are, therefore, capable of withstanding rather severe general epidemics with relatively little apparent damage. In some cases this type of apparent resistance was associated with extreme hairiness of the foliage, a characteristic that prevents the water droplets from coming in contact with the leaves proper and thus makes the fungus often unable to enter the plant. This is evidently another form of escape rather than true resistance. It is not known what reduces the frequency of infection in cases characterized by infrequency of infections with rapid growth of the fungus.

There were some seedlings in which the foliage infection was slight. In these the fungus failed to spread rapidly or to sporulate abundantly. Seedlings of this nature were found most commonly in the progeny of the cross (336-144) \times (336-18) and in the seedlings with foliage resistance in Classes 0 and 1 of table 3. These may be considered examples of physiological or true resistance.

Resistance in the Tubers

Effect of Maturity. Preliminary inoculation studies in the laboratory showed that the tubers of some resistant seedlings were susceptible to infection when young, but that the tubers of these seedling varieties developed resistance as they reached their maximum growth and maturity. Among the seedling varieties studied showing this type of reaction were Sebago, Seedling 336-123, Seedling 336-144, and Seedling 336-302. In 1936, nearly 100 per cent of the tubers of these seedlings became infected with blight rot when inoculated on August 10, whereas none were infected when the inoculations were made on September 15. Tubers of the Green Mountain and Irish Cobbler varieties, used as controls, were completely destroyed by the fungus in both of the tests.

The foliage of the seedlings was still green at the time the inoculations were made so that the tuber resistance could not be attributed to the hardening of the periderm associated with the death of the plant.

The study of the effect of the age of the tubers on resistance was continued in 1938, using the Sebago variety in comparison with Green Mountains. A set of tubers of each variety was inoculated on each of four dates.

The Green Mountains were very susceptible to decay on all of the four dates and practically all of the tubers became infected. The Sebago variety, on the other hand, was very susceptible on July 28, when all of the tubers were infected. By August 10 this variety had become quite resistant and only 10 per cent were infected. The tubers of this variety developed even more resistance as the season progressed and none developed blight rot when inoculated on August 20 and September 10.

The fact that rot occurred in the tubers inoculated early in the season suggests that possibly some of the cases of tuber resistance would be changed to cases of tuber susceptibility if blighted foliage were present when the tubers are young.

Morphological Resistance

Observations were made regarding the place of entrance of the fungus into the tubers. Inoculations in the laboratory show that susceptible varieties are often infected through the eyes or the "eyebrow." Some varieties often become infected through the cluster of eyes at the apex of the tuber.

Macroscopic studies have shown that the late-blight pathogen often penetrates the apparently unbroken periderm of the tubers of some varieties. The highly susceptible Green Mountain and Irish Cobbler varieties were often infected through the unbroken skin as well as through lenticels and the region of the eyes.

In contrast, the resistant varieties President, Paisley No. 2, and Ekishirazu, and some of the progenies of the crosses from these varieties, are only rarely infected directly through the unbroken periderm and seem to owe their tuber resistance in part to the presence of a thick protective periderm.

It was learned that infection in some varieties gained entrance through small regular cracks formed in the periderm. Infection through such openings occurred in some of the progenies in which Ekishirazu is a parent. It also resulted in a few seedlings obtained by crossing President and Katahdin. It was observed that some of the progenies in this cross possess a loose, rough skin through which infection may result.

Laboratory studies revealed that many of the varieties became infected with blight through lenticels in the periderm of the tubers. One hundred seedlings of the Paisley No. 2 \times Earlane cross were tested for tuber resistance in the laboratory. Fifty-one of these were susceptible to infection, and in all of these cases the fungus entered through the lenticels. Data were secured from 46 seedlings of the Paisley No. 2 \times Ekishirazu cross. Twenty-three of the 29 susceptible seedlings in this cross were infected through the lenticels.

It was shown previously in this paper that some varieties gain resistance

as the tubers mature. Since the young tubers of these varieties readily become infected through the lenticels, when inoculated in the laboratory, it may be inferred that the increase in resistance is due to a change in the lenticels. It may be added that many of the seedlings that become infected when artificially inoculated while young, do not readily contract the disease under field conditions. Whether this is because of differences in the lenticels of the young tubers in laboratory vs. field, is not known.

The structure of the lenticels of some varieties apparently renders them resistant to infection. Some varieties, including the Sebago, develop intumescences about the lenticels, which seem to block the entrance of the late blight fungus. The different seedlings also differ as to the relative number and size of the lenticels. In some the lenticels appear to be sparsely distributed and not conspicuously enlarged. The relationship of these factors to resistance has not been determined definitely.

Physiological Resistance

It was shown that there is a great difference in the rapidity of decay in the different varieties and progenies studied. The tubers of some possess physiological resistance.

In preliminary studies the tubers of several varieties with resistant foliage were inoculated with viable zoospores. One of these seedling varieties, namely, 45350, as selection from Katahdin selfed, was as readily infected as were the Green Mountain and Irish Cobbler controls. However, on cutting the tubers it was noted that the growth of the fungus was slow in this variety and limited to the region immediately beneath the periderm. The decay in the controls, in contrast, was rapid and extended throughout the entire tubers. (Fig. 2.)



FIG. 2. Difference in growth of late blight fungus in tubers. Above, badly decayed Green Mountain control; below, resistant S 45350, with the growth of the fungus limited to the area immediately beneath the periderm.

This subject was given additional study. Freshly dug tubers of Irish Cobblers, Green Mountains, Seedling 45350, and the President variety were halved lengthwise with sterile knives and placed in moist chambers. Conidia that had been washed from infected potato slices and from leaves were atomized onto the cut surfaces of these tubers and allowed to incubate in a cool cellar.

The mycelial and conidial development was abundant and profuse on the cut surfaces of the Green Mountain and Irish Cobbler tubers. The mycelium penetrated completely through the inoculated tubers and protruded from the lenticels. The fungus developed well on seedling 45350 but to a considerably less extent than on the two previously mentioned varieties. On President, the mycelium and conidia were sparsely developed, indicating that the fungus was growing in an unfavorable medium.

The laboratory studies conducted in 1937 and 1938 showed a great variation in the reaction of the different seedlings and varieties to tuber decay by the late-blight fungus. The decay varied from slow, with a sparse production of conidia, to rapid, with an abundant fructification. These variations occurred to some extent in the progeny of every cross studied.

The data in tables 1 to 3 show that a relatively high percentage of the seedlings in some of the crosses were resistant to rapid decay. It may be observed in table 1 that 11.5 and 31.4 per cent, respectively, of the seedlings of the Paisley No. 2 \times Earlane and the Paisley No. 2 \times Ekishirazu crosses were resistant to rapid decay, although infection occurred. Thirty per cent of the seedling progeny of Richter's Jubel \times Seedling 44537 recorded in table 2 were resistant to rapid decay after having become infected, as were also 27.2 per cent of the progeny of the Hindenburg \times Katahdin.

The nature of the resistance, just referred to, is not known. In some cases it is, no doubt, of a physiological nature; in others, it may be attributable to the cell structure of the tuber. It was noted that in some cases resistance to rapid decay was characteristic of tubers having a very firm flesh. It is possible that the fungus finds it difficult to penetrate the tubers of some varieties because of the firm nature of the flesh.

MAINTENANCE OF RESISTANCE

The question arises as to whether or not varieties now resistant to late blight will appear to become gradually susceptible. It is commonly thought that new physiologic races of *Phytophthora infestans* may be introduced or evolved that are more virulent than those now present, and that these new forms will attack the resistant varieties of potatoes that are developed.

The studies of Reddick and Crosier (2) indicate that physiologic races of *Phytophthora infestans* are not present in North America. Reddick and Mills (3) have shown that the virulence of *P. infestans* may be increased by passage through certain resistant varieties. The virulence of the pathogen was increased by successively passing a culture through the varieties Evergreen, President, and seedling K B/5. By this method the virulence

was stepped up to a point where a number of normally immune varieties were blighted badly. This building up of the virulence in *P. infestans* occurred under natural field conditions, as well as in greenhouse experiments. The present writers have grown President, Paisley No. 2, Paisley No. 3, and 5 resistant seedlings for a period of 10 years under conditions favorable for blight.⁴ None of these varieties appears to have lost its resistance to late-blight infection during this period. The Sebago variety has been grown in nonsprayed plots in Maine since 1932. This variety also had not lost its resistance in these tests. Furthermore, the writers have seen very few tubers of any of the above-mentioned varieties that were infected with the blight fungus under natural conditions. The susceptible varieties grown in the same plots have developed decay in from 10 to 75 per cent of the tubers, Green Mountains ranging from 20 to 70.

The senior writer, in the studies reported here, attempted to determine whether the late-blight organism from the resistant varieties President and Sebago was more virulent on normally resistant tubers than was the pathogen from the susceptible Green Mountain variety.

The variety President, with its resistant tubers and moderately resistant foliage, was included in Classes 1 and 2 of table 3. The foliage of Sebago is somewhat less resistant than President and generally has been included in Class 2, while the foliage of Green Mountain is very susceptible and has almost always fallen in Class 4. Both President and Sebago have resistant tubers, and the tubers of Green Mountain are very susceptible.

Late blight was very severe in Aroostook County, Maine, in 1938, and the nonsprayed plots of the susceptible variety Green Mountain were rapidly destroyed and produced small yields. Both the President and Sebago varieties gradually became infected as the season progressed, but not to the extent of the commonly grown susceptible varieties; and a relatively good crop was obtained. Figure 3 shows Sebago and Green Mountain varieties grown in alternate rows under conditions of the very severe late-blight epidemic in 1938. Under these conditions the Sebago variety was fairly resistant to infection.

The presence in 1938 of an abundance of late-blight inoculum made it easy to compare the relative virulence of the fungus from the 3 mentioned varieties. Conidia from each of the 3 varieties were washed from infected leaves and used to inoculate the tubers from all 3 varieties under laboratory conditions. The method used was that previously described. The inoculations were made late in the growing season at a time when the fungus should have had enough time in which to build up its virulence. It is possible that if several passages of the fungus through the Sebago and President could have been made before making the tests that more rot

⁴ The senior writer in 1931 secured 8 blight-resistant varieties from James K. Paisley of Albert, New Brunswick, Canada. One of them was designated as "40 Fold" by Mr. Paisley, and has been raised in Maine under the name "Rust Proof." This variety appears to be similar to the variety President. The other varieties, namely, Paisley No. 2, Paisley No. 3, and 5 other seedlings used in these studies were developed by Mr. Paisley from seed balls of his "40 Fold."

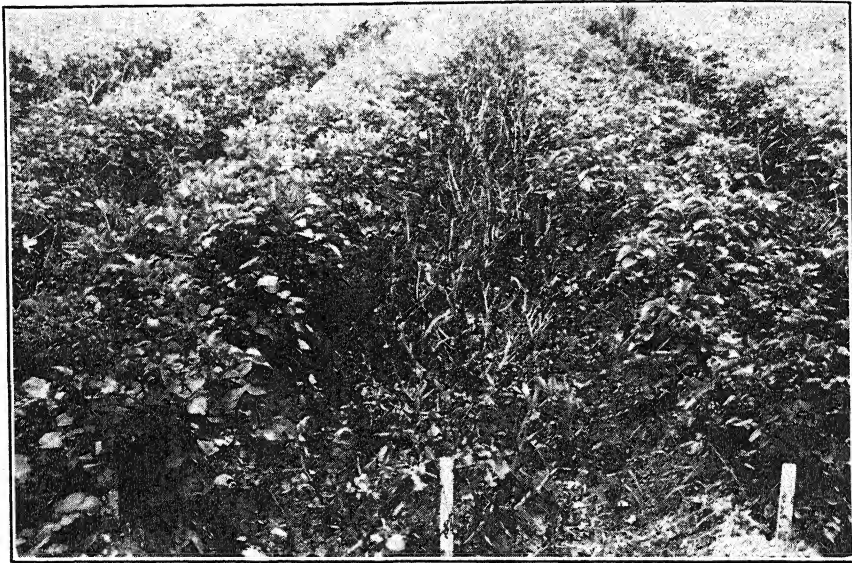


FIG. 3. Resistance of Sebago to foliage infection by late blight in 1938. Dead rows are of the Green Mountain variety, which is very susceptible.

would have resulted. It is, however, very doubtful if a more favorable season for frequent natural reinfection by late blight will occur than that of 1938. The weather conditions were favorable for late blight the entire season and were ideal for a test of this nature. The results of this experiment are given in table 4.

TABLE 4.—Resistance to late blight of potato tubers inoculated with zoospores from varieties with resistant foliage and from Green Mountains

Variety inoculated	Source of inoculum	Tuber infection ^a	Extent of decay
		<i>Per cent</i>	
Green Mountain	Green Mountain	98	Complete and rapid
Sebago	do.	4	Slight and slow
President	do.	6	do.
Green Mountain	President	97	Complete and rapid
Sebago	do.	6	Slight and slow
President	do.	4	do.
Green Mountain	Sebago	100	Complete and rapid
Sebago	do.	5	Slight and slow
President	do.	4	do.

^a The inoculations were conducted in moist chambers under laboratory conditions. Each percentage is based on readings of 100 inoculated tubers.

It may be observed from the data in table 4 that the Green Mountain tubers decayed badly as a result of the inoculations and that nearly 100 per cent were destroyed by late-blight rot, as a result of these inoculations. The Sebago and President varieties, in contrast, were resistant, and only

from 4 to 6 per cent became infected. Figure 4 depicts the contrast in susceptibility to late-blight decay of Green Mountain, Sebago, and President after being inoculated in the laboratory. The decay in the resistant varieties was relatively slow to develop. Furthermore, there was no evidence that the inoculum secured from Sebago and President was more virulent than that from the more susceptible variety, Green Mountain. These data do not support the theory that the now resistant varieties will become attacked by strains of the fungus that have been increased in virulence by passage through resistant varieties under field conditions in Aroostook

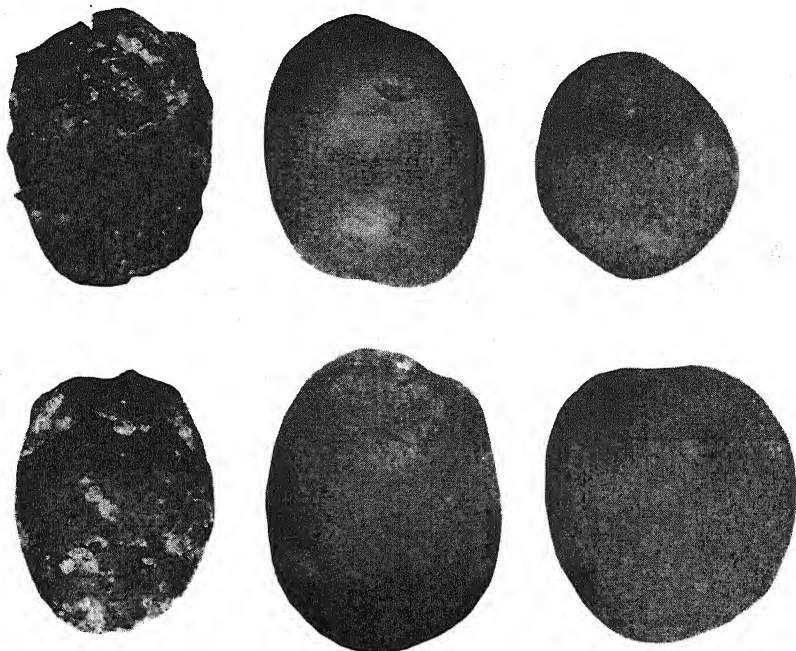


FIG. 4. Resistance of tubers to late blight. Decayed tubers at left are Green Mountain controls; sound tubers in center are Sebago and tubers at right are President. All tubers surface inoculated with spores of *Phytophthora infestans* in the same moist chamber.

County. It might be noted in passing that even if the virulence of the organism be increased on a resistant vine, the new form would not be carried over if the tubers do not become infected.

SUMMARY

Late blight caused by *Phytophthora infestans* is one of the limiting factors in potato production. Great losses are experienced in spite of large expenditures for fungicides, labor, and equipment for combating this disease.

In this study potato seedlings of known parentage, as well as the parents of these seedlings, were tested for resistance to foliage and tuber infection by the late-blight fungus under field and laboratory conditions. A high

percentage of the seedlings were resistant to tuber and foliage infection when one or both parents were resistant. Resistance in the foliage, however, was not necessarily accompanied by resistance in the tuber. Some seedlings with resistant foliage had tubers that were susceptible to infection. Other seedlings bore susceptible foliage and resistant tubers. These facts indicate that foliage resistance and tuber resistance are conditioned by different genetic factors.

Some seedlings with resistant tubers and foliage originated from selfing the susceptible variety Katahdin, showing that this variety has genotypical factors for resistance to late blight, although it is phenotypically susceptible.

Some varieties escape foliage infection because of certain growth habits or because of certain morphological structures of the leaves. Resistance in the tuber in some cases apparently depends upon the morphological structure of the periderm or of the lenticels. In other cases resistance in tubers and foliage seemed to be physiological in nature, the pathogen fruiting sparsely and growing slowly after infection.

The tubers of some of the varieties studied are, while immature, susceptible to infection by late blight, but become more resistant as they mature.

Artificial inoculations with zoospores from the varieties President and Sebago, which have blight-resistant foliage, did not infect the tubers of certain resistant varieties more readily than did zoospores from the susceptible Green Mountain variety. It did not appear that the virulence of the late-blight fungus had been increased in nature by being propagated on these resistant varieties.

The President, Sebago, and several seedling varieties have been grown for a period of 10 years in Aroostook County with no evidence that they have lost their resistance to late-blight infection. Very few tubers of these varieties have been observed to decay under natural field conditions. The Green Mountains grown in the same experiments have developed decay in from 20 to 70 per cent of the tubers.

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SOIL SICKNESS OF FLAX IN NORTH DAKOTA¹

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INTRODUCTION

The existence of a "soil sickness" complex in flax has long been recognized, but it was not until the last decade of the 19th century that the wilt phase of this complex was differentiated as a distinct disease (1, 6). Since then the major attention of investigators in the United States has been directed toward wilt to the comparative neglect of the other phases of the problem. However, the observations of Bolley and Manns (2), Boyle (3), Brentzel (4), Tervet (11), and Vanterpool (12) in North America and of European investigators, as reviewed by Schilling (10), concerning the prevalence and pathogenicity of species of *Alternaria*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Helminthosporium*, *Ophiobolus*, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Thielavia* tend to substantiate Tervet's statement that "a 'soil sickness' complex can be taken as an established fact." Except for field observations, most of the data establishing the pathogenicity of these organisms have been obtained from experiments in which flax was grown in the greenhouse during the winter months when an abnormal relation exists between light and temperature.

Data concerning the relative importance, under field conditions, of *Fusarium lini* Bolley, the wilt fungus, and the other root-rotting organisms are limited. Tervet (11) inoculated field plots with pure cultures of *Chaetomium* spp., *F. lini*, *Helminthosporium sativum* P. K. and B., *Rhizoctonia* spp., *Thielavia basicola* Zopf, and *Trichoderma lignorum* (Tode) Harz., but obtained no evidence of seedling or root injury at any stage of plant development. Boyle (3), although offering no substantiating experimental data, suggests that root-rotting fungi or cortical invaders, as distinguished from the wilt-producing vascular invaders, may infest the soil and render it unfit for crop production and may play a rôle in the occurrence of "late wilt", a condition in which the disease symptoms do not become apparent until after midseason.

The seedling-blight and root-rotting fungi usually are considered amenable to seed treatment with protective dusts. Seed treatment of flax was of little benefit to either stand or yield in tests conducted from 1931 to 1934, inclusive, at agricultural experiment stations and substations in Minnesota, Montana, North Dakota, and South Dakota (7). Extensive flax seed-treatment tests have been conducted by the writer from 1935 to 1939, inclusive, at Fargo, N. Dak., on both wilt-sick soil and on soil in the regular

¹ Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

² Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

crop-rotation plots. No significant increases in either stand or yield have been obtained by treatment with Ceresan, copper carbonate, or formaldehyde. During the period of these tests injury caused by seedling blight or damping-off fungi has been rarely observed.

Although the fungi usually associated with the "soil sickness" complex of flax, other than *Fusarium lini*, apparently caused little direct injury in the field, it was possible that they were instrumental in bringing about the condition known as "late wilt" by (1) rotting the roots and causing premature ripening, (2) attacking the plant and providing avenues of entrance for *F. lini*, (3) weakening the plant so that, although normally resistant, it would be susceptible to *F. lini* in its weakened condition. It seemed desirable, therefore, to obtain information concerning the organisms parasitizing flax roots during the early stages of growth on a flax-sick soil under field conditions, and to determine the effect of a partial restoration of a biological balance in a steamed soil on the pathogenicity of the root-rot and seedling-blight fungi.

EXPERIMENTAL RESULTS

Isolations from Field-Grown Flax Seedlings

The flax plants for the root cultural studies were grown on Plot 30 of the North Dakota Agricultural Experiment Station fields. This plot is thoroughly "flax sick," having been cropped to flax almost continuously since 1893 (2). The following varieties, possessing varying degrees of wilt resistance, as noted, were sown on May 30 and July 6, 1938, for these tests:

Bison (C.I.³ 389), highly resistant to both early and late wilt, even on Plot 30;

Rio (C.I. 280), resistant in commercial fields, but developing considerable late wilt on Plot 30;

Linota (C.I. 244), resistant in commercial fields, but wilting throughout the season on Plot 30;

Damont (C.I. 3) or Newland (C.I. 188). Both are susceptible to wilt in commercial fields. On Plot 30 few plants live longer than 2 or 3 weeks, and no seed is matured.

The seedlings were dug at successive intervals of 2 or 3 days, washed in tap water, immersed for 10 minutes in a 50 per cent saturated aqueous solution of calciumhypochlorite, plated directly on potato-dextrose agar in Petri dishes, and incubated at room temperature for 7 to 10 days.

Seed Sown May 30. Seed of Bison, Linota, and Damont was sown at a depth of approximately 1 inch at the rate of 100 seeds per 5-foot row. Soil-moisture conditions were conducive to rapid germination. Air temperatures were moderate during the period of this test, the high being 32° C. (89° F.)⁴ on June 5. The daily soil-temperature range at the 2-inch horizon for the duration of this test is shown in figure 1. The average daily

³ C.I. refers to accession number of the Division of Cereal Crops and Diseases.

⁴ U. S. Weather Bureau, Moorhead, Minn.

maximum and minimum were 24° and 14° C., respectively, considerably below the optimum for wilt as determined by Jones and Tisdale (8), who found that the minimum or "critical" soil temperature for wilt development was about 14° C., the maximum between 34° and 38° C., and the optimum between 24° and 28° C.

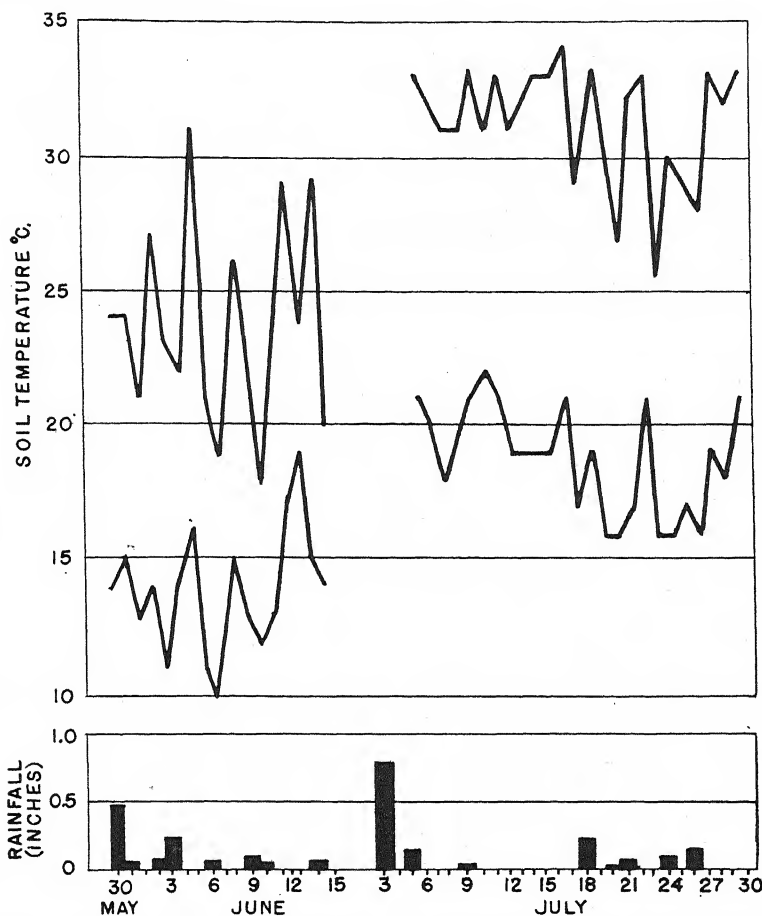


FIG. 1. Daily maximum and minimum soil temperatures at the 2-inch soil horizon on Plot 30, and precipitation at Fargo, N. Dak., during the periods May 30 to June 15 and July 3 to July 30, 1938.

Plants from each variety were dug and plated 3, 5, 8, 11, 14, and 17 days after seeding date (Table 1).

The plants dug 3 days after sowing were 1 to 2 inches long, including the tap root, but the cotyledons had not emerged above ground. These plants were cultured whole, and not one of the 179 cultured developed either bacteria or fungi. The fact that the taproot of some of these plants continued to elongate and to develop lateral roots on the culture medium after being dug, washed, and treated in the calciumhypochlorite disinfectant indicated that the method used was not unduly severe.

TABLE 1.—Results of culturing, on potato-dextrose agar, entire seedlings or segments of *Bison*, *Linota*, and *Damont* flax plants grown in infested soil for 3 to 17 days. Seed sown May 30, 1938

Segment cultured	Number of segments of <i>Bison</i>				Number of segments of <i>Linota</i>				Number of segments of <i>Damont</i>			
	Remaining sterile	Developing colonies of:			Remaining sterile	Developing colonies of:			Remaining sterile	Developing colonies of:		
		Fusarium	Bacteria	Miscellaneous fungi		Fusarium	Bacteria	Miscellaneous fungi		Fusarium	Bacteria	Miscellaneous fungi
Entire plant	52	0	0	0	62	0	0	0	65	0	0	0
Plants cultured 3 days after sowing												
Upper hypocotyl	18	0	0	0	6	0	0	0	20	0	0	0
Lower hypocotyl	56	0	0	0	57	0	0	0	65	0	0	0
2nd inch of root	18	0	0	0	6	0	0	0	20	0	0	0
Root tip	18	0	0	0	6	0	0	0	20	0	0	0
Plants cultured 5 days after sowing												
Upper hypocotyl	39	0	0	0	30	0	0	0	30	0	0	0
Lower hypocotyl	40	0	0	0	32	0	0	0	31	0	0	0
2nd inch of root	39	0	0	0	23	0	0	0	26	0	0	0
3rd " " "	34	0	0	0	7	0	0	0	16	0	0	0
4th " " "	28	0	0	0	2	0	0	0	9	0	0	0
5th " " "	22	0	0	0
Plants cultured 8 days after sowing												
Upper hypocotyl	33	1	5	0	33	3	6	0	18	6	4	0
Lower hypocotyl	41	7	2	0	33	6	3	0	18	9	0	0
2nd inch of root	44	1	0	0	15	6	8	2 ^{a, b}	15	5	1	0
3rd " " "	25	2	2	1 ^b	8	1	0	1 ^a	11	2	1	1 ^d
4th " " "	17	0	0	0	4	0	0	0	7	2	0	1 ^a
5th " " "	15	1	0	0	1	0	0	0
6th " " "	8	0	0	0
7th " " "	2	0	0	0
Plants cultured 11 days after sowing												
Upper hypocotyl	52	0	3	0	7	0	11	0	7	2	4	0
Lower hypocotyl	51	3	1	0	17	0	2	0	11	1	3	0
2nd inch of root	54	0	0	0	15	1	0	0	7	1	1	0
3rd " " "	26	1	1	1 ^c	10	0	0	0	3	0	0	1 ^d
4th " " "	15	0	0	0	6	0	0	0	2	0	0	0
5th " " "	9	0	0	0	6	0	0	0
6th " " "	6	0	0	0
Plants cultured 14 days after sowing												
Upper hypocotyl	18	1	1	0	17	1	2	1 ^c	16	12	6	0
Lower hypocotyl	10	7	6	1 ^d	16	3	0	1 ^a	9	14	5	0
2nd inch of root	11	3	2	4 ^d	16	0	0	0	5	7	7	0
3rd " " "	7	1	2	3 ^d	9	0	0	0	5	3	1	2 ^d
4th " " "	5	0	1	2 ^d	3	0	0	0	4	0	0	0
5th " " "	5	0	0	1 ^d	2	0	0	0	1	0	0	0
Plants cultured 17 days after sowing												

^a *Thielavia basicola*.

^b *Rhizopus* sp.

^c *Alternaria* sp.

^d Sterile fungus.

The plants dug 5 days after sowing were 3 to 5½ inches long, including the taproot, and the cotyledons were just breaking the surface soil. From this and subsequent diggings only that portion of the plant below the cotyledons was cultured. It was difficult to obtain whole plants after the 5th day because of the rapid elongation of the root in the heavy clay soil. As large a portion of each plant as possible was obtained and cut into segments approximately an inch long, as follows: (1) the stem portion of the upper hypocotyl just below the cotyledons, (2) the lower portion of the hypocotyl and upper root, and (3) each inch of root below the hypocotyl. No fungi nor bacteria developed on any plant segment dug and cultured on the 5th or 8th day after sowing.

So much wilt had developed in the Damont variety 11 days after sowing that only about half the plants were satisfactory for culturing. Colonies of *Fusaria* and bacteria developed from cultured segments of all 3 varieties in each series dug 11, 14, and 17 days after sowing. Many of the bacterial colonies were on segments bearing a *Fusarium* colony and were probably secondary organisms. Most of the colonies of *Fusaria* developed from the hypocotyl. The low proportion of upper hypocotyl segments of Bison cultured 11, 14 and 17 days after sowing that developed colonies of *Fusarium* (2 of 105) in contrast to Damont (20 of 61) may indicate the ability of Bison to localize the infection. Only 3 of the fungi supposedly associated with the "soil sickness" complex of flax were obtained in this series of tests. Two colonies of *Alternaria* spp. and 4 of *Thielavia basicola* developed on cultured segments. Colonies of *Fusarium* spp. developed from 113 segments of which 28 were from Bison, 21 from Linota, and 64 from Damont.

Seed Sown July 6.—In the series sown July 6, Bison, Rio, Linota, and Newland seed was sown in moist soil favorable for rapid germination. Seasonally moderate temperatures prevailed during the period of this test, the maximum air temperature was 34° C. (93° F.) recorded on July 12 and 15. The soil temperature at the 2-inch horizon was higher than in the earlier sowing (Fig. 1) and more favorable for wilt development; the average daily maximum was 31° C., and the average daily minimum was 19° C. Considerable heat canker developed despite the moderate temperatures. This may be explained by supplementary temperature readings made July 12 at 3 p.m., when the air temperature was 34° C. The temperature of the soil at the surface, with the bulb of the thermometer just covered with soil, was 57°, at the 1-inch horizon, 48°, and at the 2-inch horizon, 33° C.

Because of higher soil temperature both the flax plants and the wilt developed more rapidly in the July 6 than in the May 30 sowing. In 3 days the plants were 3 to 4 inches long, taproots included, and the cotyledons were breaking the surface; in 5 days they were 6 to 7 inches long. In this test, the entire plant, or as much of it as was obtained, was cultured as a unit. This included the entire hypocotyl and from 1 to 9 inches of root. The data obtained from culturing plants dug at 2- or 3-day intervals for 24 days after sowing are given in table 2.

TABLE 2.—Results of culturing, on potato-dextrose agar, flax plants grown in "flax-sick" soil for 3 to 24 days. Seed sown July 6, 1938

Variety and C.I. No.	Age of cultivated seedlings in days	Wilt in row dug ^a (Per cent)	Number of plants cultured							
			Total	Remained sterile	Developed colonies of:					
					Fusarium	Bacteria	Trichelia	Rhizoctonia	Alternaria	Miscellaneous fungi
Bison (C.I. 389)	3	78	78
	5	69	69
	7	64	60	4
	9	9	45	36	7	2	1
	12	28	34	26	7	1
	14	28	22	10	11	1
	16	22	29	11	15	2	1
	19	37	16	21
	21	30	14	14	3
	24	36	10	19	6	1	1
Newland (C.I. 188)	3	55	55
	5	72	72
	7	69	64	5
	9	46	60	43	17
	12	92	39	19	20
	14	97	31	26 ^b	4	1
	16	93	24	12 ^b	12
	19	34	32 ^b	2
	21	25	24 ^b	1
	24	28	25 ^b	3
Linota (C.I. 244)	3	85	85
	5	70	70
	7	56	55	1
	9	32	57	36	21	2
	12	53	26	16	8	2
	14	60	22	15 ^b	7
	16	76	34	18 ^b	13	2	2
	19	24	15 ^b	9
	21	36	16 ^b	16	1	2	2
	24	27	21 ^b	3	2	2
Rio (C.I. 280)	3	56	56
	5	62	62
	7	62	62
	9	26	47	46	1
	12	28	26	21	5
	14	23	34	17	14	2	1
	16	17	22	7	12	1	4
	19	35	15	16	1	3	2
	21	27	11	12	2	3
	24	25	8	12	3	4
Total	1714	1354	312	29	8	4	13	9

^a Because of the small size of the plants and the rapidity with which they dried after dying, it was difficult to differentiate between those dead from heat canker and from wilt. Most of the dead plants of Bison and Rio probably had been killed by heat canker.

^b Most of these plants remaining sterile had been dead for some time and were dried.

Neither fungi nor bacteria developed on any of the 547 plants dug and cultured after 3 and 5 days' growth on Plot 30. Of 251 plants cultured after growing 7 days on Plot 30, 241 were sterile and only 10 developed *Fusarium*. After 9 days' growth, 161 plants of 209 cultured remained sterile, 46 developed colonies of *Fusarium*, 2 developed bacterial colonies, and 3 developed colonies of fungi not reported as pathogenic to flax. For the test as a whole, 1,354 plants of the 1,714 cultured were sterile. The 360 diseased plants developed colonies as follows: 312, *Fusarium* spp.; 13, *Alternaria* spp.; 8 *Thielavia basicola*; 4, *Rhizoctonia* spp.; 9, other fungi not reported as pathogenic to flax; and 29, bacteria. Most of the colonies of *Alternaria* spp. developed at the severed stem end and were probably not due to soil infection and not important in the "soil sickness" complex. All of the colonies of *T. basicola* developed on Rio, the variety most subject to late wilt. However, since infections of Rio with *Fusarium* spp. were more numerous and occurred earlier than infections with *T. basicola*, there was no basis to assume that the latter had in some way paved the way for infections by *Fusarium* spp. These studies indicate that Rio is more resistant to initial infection by *Fusarium* spp. than is the more wilt-resistant Bison variety. Rio produces a large taproot that may resist attacks by *Fusarium* spp. longer than the smaller roots of the other varieties.

Also recorded in table 2 are the percentages of wilted and dead plants in the rows dug 9, 12, 14, and 16 days after the July 6 sowing. The fact that the percentage of wilted and dead plants of Bison and Rio remained essentially unchanged after the 12th day indicates that injury to these varieties was largely the result of heat canker. There was considerable heat-canker injury to Linota and Newland also, but 26, 53, 60, and 76 per cent of dead and wilted plants in Linota and 46, 92, 97, and 93 per cent in Newland at the end of 9, 12, 14, and 16 days, respectively, are indicative of the progressive effect of wilt.

The small number of isolates of *Fusarium* spp. obtained from the plants of Linota and Newland cultured during the latter part of the test, in contrast to the high percentage of dead and wilted plants in these varieties, probably was attributable to culturing of thoroughly dried plants that had been killed by heat canker or wilt early in their development.

Cultural Study of Partly Wilted Plants. On June 30, 1939, 8 plants showing symptoms of late wilt were selected at random from Plot 30. The lower leaves on the main stem of these plants were turning yellow and falling off and the terminal leaves, although green, were flaccid. Each of these plants bore apparently normal basal branches from the cotyledonary node or crown. Tissue segments from parts of plants enumerated in table 3 were cultured as in the preceding studies.

Fusarium lini was the only fungus that developed from any of these segments. The high proportion of *F. lini* colonies that developed on all segments from the roots and lower portion of the main stem would indicate systemic infection. Symptoms of wilt and infection by *F. lini* were not

absolutely coincident, since the fungus developed from only 1 of 8 stem segments taken just below the flaccid tip of the wilted stem and from half of the stem segments of apparently healthy branches taken 1 inch above the crown. The apparently healthy branches were free from the fungus near their tips, since none of the segments taken 3 inches above the crown developed *F. lini*.

TABLE 3.—*The distribution of Fusarium lini in partly wilted plants grown on Plot 30 as indicated by tissue culture studies*

Portion of plant cultured	Number of segments cultured	Number of segments developing <i>Fusarium lini</i>
Root 3 in. deep	8	8
Root 1 in. deep	8	7
Cotyledonary node	8	7
Wilted stem 1 in. high	8	7
Wilted stem 3 in. high	8	7
Wilted stem 5 in. high	8	1
Normal stem 1 in. high	8	4
Normal stem 3 in. high	8	0

POT TESTS WITH PYTHIUM SPP. AND RHIZOCTONIA SPP.

Three of the fungi associated with the "soil sickness" complex of flax (3, 11) have proved destructive under certain conditions at Fargo. Injury by *Fusarium lini* has been amply demonstrated (1, 2, 3, 11). A species of *Rhizoctonia* has been observed causing occasional seedling blight in the field, and a species of *Pythium* caused damping-off of flax in the greenhouse. A pot experiment was conducted to determine the pathogenicity of the 2 last-named fungi to successive plantings of Bison flax in Fargo clay field soil (not "wilt-sick") and in a similar soil that had been steamed for 2 hours. The inoculum, grown on potato-dextrose agar in Petri dishes, was incorporated with the upper 2 inches of soil immediately preceding the first sowing. Potato-dextrose agar was added similarly to the control pots.

TABLE 4.—*Percentage survival of successive plantings of Bison flax in pots of nontreated and steamed Fargo clay soil, inoculated with Pythium spp. and Rhizoctonia spp. isolated from flax, Fargo, N. Dak., 1939*

Soil treatment	Inoculation	Survival of seedlings:		
		Sown March 6, observed April 4	Sown April 7, observed May 2	Sown May 15, observed May 27
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Nontreated	Control	82	88	97
Nontreated	<i>Pythium</i> spp.	85	96	92
Steamed	Control	96	75	97
Steamed	<i>Pythium</i> spp.	25	70	90
Nontreated	Control	88	100	97
Nontreated	<i>Rhizoctonia</i> spp.	5	76	100
Steamed	Control	97	97	87
Steamed	<i>Rhizoctonia</i> spp.	20	67	20

Four pots of each treatment were used. The survival of successive greenhouse plantings made in March, April, and May, 1939, is given in table 4.

Pythium inoculation of nontreated farm soil resulted in no apparent injury in any of the plantings, even in the one made immediately after applying the inoculum. *Pythium* inoculation of steamed farm soil resulted in severe preemergence and damping-off injury and the stunting of surviving plants, in the series sown immediately after applying the inoculum (Fig. 2, A).

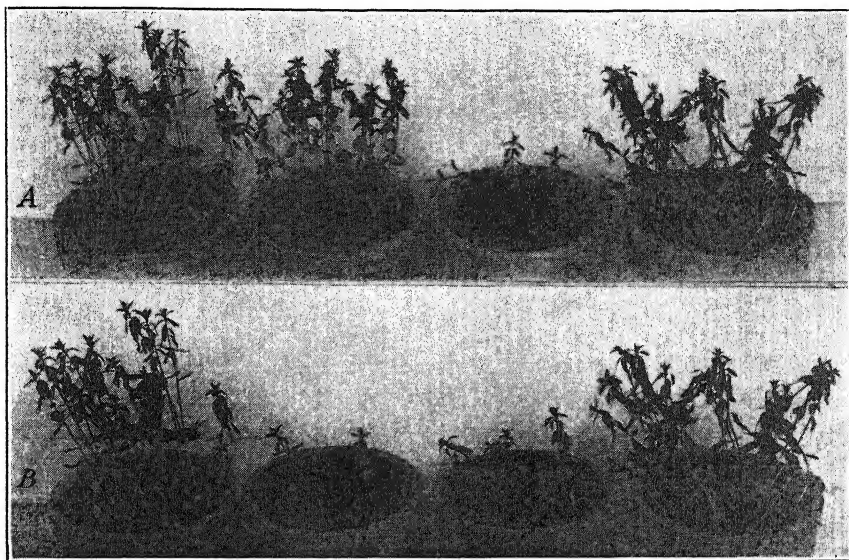


FIG. 2. Virulence of *Pythium* spp. and *Rhizoctonia* spp. in Fargo clay soil sown with Bison flax immediately following soil inoculation. A. Left to right, field soil not steamed, not inoculated; field soil not steamed, inoculated with *Pythium* spp.; field soil steamed and inoculated with *Pythium* spp.; and field soil steamed, not inoculated. B. Left to right, field soil not steamed, not inoculated; field soil not steamed, inoculated with *Rhizoctonia* spp.; field soil steamed, inoculated with *Rhizoctonia* spp.; and field soil steamed, not inoculated.

There was considerable injury to the second sowing made April 7 in the *Pythium*-inoculated steamed soil, evinced by a stunting of the plants and a rotting of the roots, especially the lateral roots. In this series the plants growing in the noninoculated steamed soil were stunted and developed more root rot than those growing in either the *Pythium*-inoculated or the nontreated soil. This may have been due to the infestation of the steamed soil by organisms that did not thrive in a soil of more normal biological balance. There was little difference in rate of growth or incidence of root rot of flax plants sown May 15 in either of the *Pythium*-inoculated or the noninoculated soils.

Rhizoctonia was more destructive than *Pythium* in both the steamed and nontreated soils. Preemergence and seedling-blight injury were severe in both *Rhizoctonia*-inoculated soils sown immediately after inoculation.

(Fig. 2, B.) This injury persisted in a more moderate form in the second series, sown April 7. No injury either in the form of a stunting of the plants or as root rot occurred in the third series sown May 15 in non-treated soil. Plants growing in the steamed soil that had been inoculated with *Rhizoctonia* were more severely injured in the third sowing than in the second. The maggots of fungus gnats *Sciara* spp. were found in this soil, feeding upon the rotted flax roots. The association of these maggots with decaying flax roots has been frequently encountered by the writer in wilt and root-rot studies made in the greenhouse in which steamed soil has been used.

DISCUSSION AND CONCLUSION

In a study of the pathogenicity of root-rotting and damping-off fungi consideration should be given to the rôle of antibiosis and related phenomena on the causal organisms and to the effect of environment on the morphology and physiology of the host. The results reported in this paper, showing that certain fungi that cause severe damping-off or seedling blight when tested in the greenhouse in steamed soil but are innocuous or rapidly lose their virulence in normal field soil, are not unique, Sanford (9), studying wheat root rots in Alberta, reported failure to obtain satisfactory artificial infestation of field soil with *Ophiobolus graminis* Sacc., *Helminthosporium sativum*, and *Fusarium culmorum* (W. G. Sm.) Sacc. Broadfoot (5) found that *O. graminis*, when just introduced into steam-sterilized soil, was highly virulent on wheat, but that its virulence decreased rapidly when such artificially inoculated soil was left to natural contamination. Tervet (11) found certain strains of *H. sativum*, *Rhizoctonia* spp., and *Pythium* spp. highly virulent to seedling flax in greenhouse tests in steamed soil. He also obtained injury by these organisms and by *Thielavia basicola*, *Ophiobolus graminis* Sacc. (*O. cariceti* (Berk. and Broome) Sacc.), *Alternaria* spp., and *Fusarium lini* in field soil in greenhouse tests but observed no indication of injury to flax grown in field plots inoculated with these fungi. Boyle (3) observed that roots of flax plants grown in the greenhouse during the winter were very different from those grown in the field during the spring and summer. He noted that the root of a flax plant grown in the field for 20 days was about twice the size of a root grown in the greenhouse for 31 days and had a well-developed, heavily-suberized endodermis in contrast to only a trace of suberization in the root grown in the greenhouse.

Determinations of the pathogenicity of soil fungi producing root rot or damping-off of flax seedlings based on greenhouse tests in steamed soil are of little value as a measure of the destructiveness of these organisms under field conditions. In such tests not only does the fungus have no competition with other organisms universally present in normal farm soils but the host is grown under abnormal conditions that greatly prolong the period during which the root tissues are in a succulent stage and, therefore, highly susceptible to injury by root-rotting organisms. The culture studies,

reported in this paper, of flax varieties grown for periods of from 3 to 24 days on soil that had been cropped almost continuously to flax for 45 years indicated that seedling blight and damping-off were of little importance. Although preemergence injury is a striking feature of greenhouse tests with the common root-rotting organisms, none occurred in these field trials. As a matter of fact, the tissue-culture studies indicated that even the highly wilt-susceptible varieties Damont and Newland had not become infected with *Fusarium lini* until after emergence of the cotyledons.

Of the organisms reported as responsible for seedling blight of flax (10, 11), *Fusarium lini*, *Rhizoctonia* spp., *Alternaria* spp., and *Thielavia basicola* were isolated from roots of field-grown seedling flax plants. No difficulty has been experienced, when the same isolation technique was used in reisolating *Helminthosporium sativum* and *Pythium* spp. from greenhouse experiments in which they have been highly virulent. *Alternaria* spp. and *T. basicola* caused little seedling injury in soil-inoculation studies in the greenhouse and none in the field.

The writer has observed only one instance of serious seedling-blight injury in western Minnesota and eastern North Dakota during the period 1931 to 1939. The stand of Bison flax, in a seed-treatment test sown at Fargo on noninfested farm soil in 1935, was materially reduced by seedling blight caused by a species of *Rhizoctonia*. None of the treating materials used (Ceresan, copper carbonate, or formaldehyde) was effective in reducing the injury.

The prevalence of *Fusarium lini* and the paucity of other flax root-rotting fungi in isolations made from roots of wilt-resistant as well as wilt-susceptible varieties of flax grown for periods of from 3 to 24 days in a thoroughly "flax-sick" soil indicate that the "soil sickness" complex of flax at Fargo is primarily attributable to wilt. This is further borne out by (1) the failure to observe serious outbreaks of seedling blight or damping-off in the field, (2) the failure of seed treatments to increase stand or yield, and (3) the transitory or relatively weak pathogenicity of such soil-borne flax root-rotting fungi as *Pythium* spp. and *Rhizoctonia* spp. in normal farm soil.

SUMMARY

A study was made of the progress of root infection of seedling flax plants growing in "flax-sick" soil in the field as indicated by the developing of fungi from roots and hypocotyls dug at 2- or 3-day intervals and plated on potato-dextrose agar in Petri dishes.

No preemergence injury occurred in the field and no fungi developed from the flax plants until well after emergence of the cotyledons.

Of the soil-borne organisms considered to be involved in the "soil sickness" complex of flax, *Fusarium lini*, *Thielavia basicola*, and species of *Alternaria* and *Rhizoctonia* were obtained in these tests. The earliest infection of both wilt-resistant and wilt-susceptible varieties was by *Fusarium lini*. This fact, together with the preponderance of isolates of

F. lini (425 as compared with 15 of *Alternaria* spp., 16 of *T. basicola*, and 4 of *Rhizoctonia* spp.), indicates that *F. lini* was primarily responsible for the injury.

Severe seedling injury was obtained in greenhouse tests in both steamed and nonsteamed soil when sown immediately after inoculation with *Rhizoctonia* spp. Injury diminished in successive monthly sowings and had practically vanished in the third sowing in nonsteamed soil.

In steamed soil damping-off was severe when flax was sown immediately after inoculation with *Pythium* spp., but injury diminished in successive monthly sowings. In nonsteamed farm soil no injury was apparent even when flax was sown immediately after inoculation of the soil.

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WILT RESISTANCE OF THE RIVERSIDE VARIETY OF TOMATO TO BOTH FUSARIUM AND VERTICILLIUM WILTS¹

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INTRODUCTION

Varieties of tomatoes fairly resistant to Fusarium wilt (*F. lycopersici* Sacc.) are now widely distributed and have been cultivated with good results for a number of years. Very few commercial varieties resistant to Verticillium wilt (*V. albo-atrum* R. and B.) are, however, available, and

¹ Paper No. 409, University of California Citrus Experiment Station, Riverside, California.

² The writers are much indebted to Dr. S. P. Doolittle for his numerous useful suggestions in the course of the preparation of this manuscript.

there are none that can be relied upon to produce a satisfactory crop on soil heavily infested with both of these fungi. Soil infestation with the latter fungus is, however, rather frequent in California tomato fields, especially along the coast, where *V. albo-atrum* is self-perpetuating on a great variety of hosts.³ Under such conditions Marglobe is apt to be severely affected by wilt, because of its susceptibility to *Verticillium*, whereas other commercial varieties less resistant to *Fusarium*, are apt to be attacked by both fungi. Consequently, an acute need has been felt for a shipping, as well as a canning, variety capable of withstanding the attack of both diseases. It is hoped that the Riverside tomato, developed and introduced cooperatively by the United States Department of Agriculture and the University of California,⁴ may help to meet this need. Absolute immunity from either *Fusarium* or *Verticillium* is not claimed for this new variety, but it is obvious from the data presented below that Riverside showed only slight injury under such conditions as induced severe damage in certain other varieties. This favorable showing, coupled with certain superior qualities of the fruit, made desirable an immediate distribution of the seed of this new variety.

METHODS

The Riverside tomato originated from a cross made in 1928 between Cal 2, and Marvana.⁴ Cal 2 resembles Santa Clara in maturing very late, the peak of harvest being about 125 days after transplanting to the field. It is well-adapted only to such localities as the coastal part of southern California, and is moderately resistant to *Verticillium* and *Fusarium* wilts. Marvana matures very early, the peak of harvest being about 94 days after transplanting. It is relatively resistant to *Fusarium*, but not appreciably so to *Verticillium*. The initial cross, Cal 2 × Marvana, was followed by repeated self-fertilization and single-plant selection for 8 successive generations. One F₉ population was named "Riverside" and seemed worthy of extensive trial, since it gave a desirable type of fruit, not unlike Norton in shape and internal structure (Fig. 1), and appeared to be very productive, especially in the coastal region.

The majority of the generations that led to the development of the Riverside tomato were grown, together with Stone, Marglobe, and, occasionally other varieties, in soil heavily infested with both the *Fusarium* and *Verticillium* wilt organisms, and their relative tolerance to these diseases has thus been ascertained. Our principal test plots were located in the coastal region of southern California, at Santa Ana, Orange County, and San Onofre, San Diego County. Some supplementary tests also were made at Riverside, where the summer climate tends to be drier and warmer. In these localities the growing season is decidedly long. Suitable ground for the test plot was selected at Santa Ana in 1929 after a preliminary planting

³ Rudolph, B. A. *Verticillium* hadromycosis. *Hilgardia* 5: 197-360. 1931.

⁴ Lesley, J. W., and M. Shapovalov. The Riverside tomato, a new variety resistant to two wilt diseases. *Seed World* 41(7): 8-9. 1937.

of 11 outstanding commercial varieties. In October of that year samples of wilted tomato plants were taken at 13 widely scattered points in the field. Five of the cultures made from this material yielded *Verticillium albo-atrum* and 8 yielded *Fusarium lycopersici*.

As the field symptoms of the two tomato wilts are very similar at certain stages of their development, external characteristics do not furnish a reliable basis for determining the causal organism involved. Reliable data could be obtained only from laboratory cultures; and this means of identification was used throughout the development of the Riverside variety,

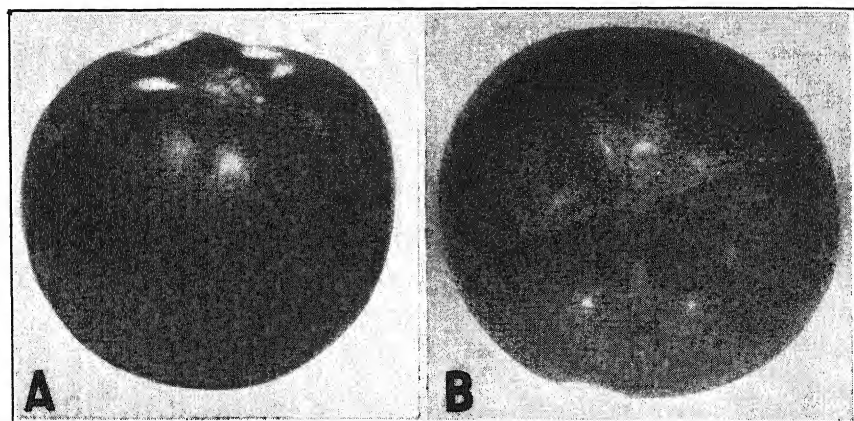


FIG. 1. A. Stem-end and side view of Riverside tomato. B. Norton tomato showing typical stem-end cracking. Both somewhat reduced.

TABLE 1.—Comparison of resistance to both *Fusarium* and *Verticillium* wilts of the Riverside tomato with other varieties under field conditions for 1938 at Santa Ana, California

Variety	Number of plants					
	Total	Healthy	W1	W2	W3	W4
Riverside, (a)	119	...	28	84	3	4
“ (b)	65	1	42	22
Total	184	1	176		7	
Total per cent	0.5	95.7		3.8	
Marglobe, (a)	78	17	36	25
“ (b)	67	...	7	40	16	4
Total	145	0	64		81	
Total per cent	0	44.2		55.8	
Stone, (a)	80	8	32	40
“ (b)	65	...	13	31	17	4
Total	145	0	52		93	
Total per cent	0	35.8		64.1	
Pearson, (a)	78	...	18	53	6	1
“ (b)	67	...	28	37	2	0
Total	145	0	136		9	
Total per cent	0	93.8		6.2	

including the plot-infection test. As a rule, cultures were made from portions of the lower branches cut close to the main stem of severely affected plants (W3 and W4, Table 1), or, if such plants were not available, of those less severely affected. No attempt was made to obtain a culture from every affected plant, but only to determine the etiological nature of the wilt in the more severely affected plants of each variety at each sampling. From 2 to 4 culture platings were made from each stem sample from different parts of the vascular tissues. Prune or potato agar usually was employed in these tests. Some of the specimens failed to yield any organism. This failure may have resulted from the fact that the discoloration of the vascular bundles often precedes their actual invasion by the parasite, as has been found by other workers in the case of various wilt-infected plants. It was thought, however, that the number of blank cultures throws additional light on the behavior of different varieties; hence, records of these are included in the tabulated results.

The severity of the disease was judged by the external symptoms, as shown by the condition of the vine, and also by the amount of fruit produced. No attention was given either to presence or intensity of vascular discoloration, the resistance of the varieties tested being evaluated on the basis of their tolerance to the disease and their ability to produce a crop, rather than on that of complete immunity.

Several observations were made during the growing season, and the condition of a plant was recorded according to the following symbols: H, a healthy or nearly healthy plant; W1, a plant showing one or very few definitely yellow leaves; W2, a plant showing several definitely yellow leaves; W3, a plant with a whole branch severely affected, and W4, a very severe, advanced stage of wilt rendering the plant wholly unproductive. The various generations from the cross Cal 2 \times Marvana in the direct ancestral line of the Riverside variety are designated simply F₃, F₄, etc., to avoid the use of unwieldy pedigree numbers.

EXPERIMENTAL

The field trials for wilt resistance were begun in 1930, with the F₃ generation of the Riverside line and several commercial varieties as checks, and concluded with F₉ generation in 1938. As the F₃ to F₈ generations may not have been homozygous for the wilt-resistance genes, the detailed results obtained with these generations are omitted from this discussion.

The 1938 data relating to the amount and severity of wilt for the principal varieties used in our tests are summarized in table 1 and represent the final results obtained on October 7. The data from the 2 adjacent portions of the field, a and b, are shown separately, since the symptoms were less severe in portion b.

As in previous preliminary tests, the Riverside variety is much more resistant to wilt than either Stone or Marglobe. It had the lowest percentage (3.8 per cent) of W3 and W4 plants, *i.e.*, cases involving stem destruction and serious reduction of yield. Marglobe and Stone developed

a high percentage (55.8 and 64.1 per cent, respectively) of severe (W3 and W4) cases and a correspondingly low percentage of mild cases (W1 and W2). It is noteworthy that Pearson also showed a fairly high resistance to wilt. This variety is an important shipper, though apparently it may contain too much hard core for canning.

Riverside yielded very well, considering the rather low level of soil fertility of the field. The yield from Pearson was equally good. Stone was severely diseased and extremely unproductive. Marglobe also was severely diseased, with foliage often a sickly yellow tinged with blue, and the yield very poor. In October, as the weather became cooler and the days shorter, both Stone and Marglobe, especially the former, made new healthy growth, and wilt symptoms were less conspicuous; neither produced a crop with this late growth.

The Riverside tomato was included in the tomato seed-source trials conducted by Harold T. Cook of the Virginia Truck Experiment Station,

TABLE 2.—*Relative resistance of the Riverside variety of tomato to Fusarium wilt under conditions of eastern Virginia*

Year	Variety	Amount of wilt	Wilt grade	Yield per acre in bushels
1937	Riverside (25 plants)	1 plant only	0.040
	21 other wilt-resistant strains and varieties including Marglobe, Pritchard, Rutgers, and others (25 to 75 plants of each) range	5 to 37 plants	0.225 to 2.200
	3 susceptible varieties Greater Baltimore, Stone and Free-state (75 plants of each) range	49–56 plants	2.130 to 2.790
1938	Riverside (25 plants)	24.0%	0.56	260.5
	30 other wilt-resistant strains and varieties, including Marglobe, Pritchard, Rutgers, Norton and others (50 to 125 plants of each) range	52 to 90.7%	1.04 to 3.11	230.0 to 345.2
	4 susceptible varieties Greater Baltimore, Stone and others (50 to 125 plants of each) range	79.6 to 97.0%	2.61 to 3.75	178.8 to 282.6
1939	Riverside (75 plants)	12%	0.20
	34 other wilt-resistant strains and varieties, including Marglobe, Pritchard, Rutgers, and others (75–125 plants each) range	28.8 to 85.3%	0.74 to 3.20
	1 susceptible variety Greater Baltimore	89.6%	3.14

Norfolk, Va., during the seasons of 1937, 1938, and 1939. According to Cook's reports, Riverside showed a higher degree of resistance to *Fusarium* wilt than any of the varieties tested. The percentage of wilt, as well as the wilt grade⁵ were lower and the yield was at least equal to the average. This may be seen from table 2, prepared for the writers by Dr. Cook.

The Riverside variety also was tested for wilt resistance (*Fusarium lycopersici*) by Young,⁶ in Texas, and proved to be one of the most resistant in his collection, when grown either in soils free from *Heterodera marioni* or in those infested with this nematode.

In order to ascertain the type of wilt that affected different varieties in different seasons in California, cultures were made several times during the summer from plants showing relatively severe symptoms of wilt. Summarized results of such cultures for 1938 are presented in table 3.

TABLE 3.—Cultures obtained from wilt-affected tomato samples in 1938. (3 cultures were made from each stem, 1 from each main vascular bundle)

Variety	Total No. of cultures	Type of culture obtained			
		<i>Verticillium</i> only	<i>Fusarium</i> only	Both <i>Verticillium</i> and <i>Fusarium</i>	No cultures
Riverside { Number	160	112	2	2	44
{ Per cent	70	1.25	1.25	27.5
Marglobe { Number	152	116	12	4	20
{ Per cent	76.4	7.9	2.6	13.1
Stone { Number	132	76	49	2	5
{ Per cent	57.6	37.1	1.5	3.8
Pearson { Number	36	25	0	0	11
{ Per cent	69.5	0	0	30.5
Total for all varieties	480	329	63	8	80
Total per cent	68.5	13.2	1.66	16.7

An examination of this table shows that *Verticillium* developed in a majority of cultures obtained from the diseased tomato stems in our plots. The initial test of the soil infection at Santa Ana in 1929 indicated the presence of both fungi in various parts of the field. In subsequent trials during the period of 1930–1934, the *Fusarium* infection was fairly high in Stone, but relatively slight in Riverside, Marglobe, and Pearson, thus suggesting a higher resistance of these varieties to *Fusarium* than to *Verticillium*. No evidence was obtained regarding the accumulated infestation of the soil.

The pure *Verticillium* infection in the above mentioned varieties was accompanied by symptoms of a predominantly mild type; but, when both

⁵ The wilt grade is based on 4 stages of the disease and calculated according to the formula: $\frac{S(w \times n)}{N}$, where S signifies summation, w the stage of the disease, n the number of plants in that stage, and N the total number of plants of the variety.

⁶ YOUNG, P. A. Tomato wilt resistance and its decrease by *Heterodera marioni*. Phytopath. 29: 871–879. 1939.

fungi were present, the disease usually showed a more severe form. This was also the case with pure *Fusarium* infection. Since the *Fusarium* was less frequent, severe cases of wilt were less common. It should be mentioned, however, that in spite of the generally mild character of the symptoms the *Verticillium* disease seemed to affect the yield of the susceptible varieties very materially. The Riverside lines, as the most resistant to both wilt fungi, gave relatively high yields.

Repeated cultural tests during the season indicated that the type of wilt present at any given time was determined not only by the fungus-host relationship, but also by the prevailing environmental conditions. With both *Fusarium* and *Verticillium* present in the soil, it was frequently observed that wilt symptoms during the hot part of the summer first appeared on many of the plants susceptible to *Fusarium*, whereas later in the season *Verticillium* wilt predominated on plants susceptible to it. This relationship between the seasonal climatic changes and the kind of wilt is what might be expected, since *Fusarium lycopersici* is known to have a higher optimum growth temperature than *V. albo-atrum*. Table 4 gives the actual relationship between the cultures of *Verticillium* and *Fusarium* in different parts of the season and in different years.

TABLE 4.—Relative prevalence of *Verticillium* and *Fusarium* in pure cultures made from wilted tomato plants at different times of the season at Santa Ana, California

Fungus	1931					1932				1932	1934				1938		
	August 10	September 1	September 17	October 8	November 3	August 26	September 2	September 27	October 19	October 13	September 1	September 6	September 27	October 15	September 12	October 7	October 25
<i>V. albo-atrum</i>	0	0	3	20	32	18	33	22	39	69	9	27	44	23	165	74	102
<i>F. lycopersici</i>	8	10	17	0	16	0	0	5	0	3	3	3	0	1	47	7	9
Both <i>Fusarium</i> and <i>Verticillium</i>	0	0	4	0	0	2	1	2	0	3	0	0	0	0	4	2	1
Total	8	10	24	20	48	20	34	29	39	75	12	30	44	24	216	83	112

All cultures were made from diseased tomato stems of the varieties listed in tables 1 and 3, as well as some other varieties grown in the same field in certain years. The majority of the samples were taken from new, definite cases of wilt as the disease was developing through the season. Occasionally, second samples were taken at a later date from plants sampled earlier, different branches being used. In a small number of cases in which *Fusarium* was isolated at the first sampling, this fungus was either absent or appeared along with *Verticillium* on the second sampling, indicating possible slowing down in the rate of development of *Fusarium* later in the season.

It is evident from table 4 that of the total of 427 cultures made in August and September, nearly 22 per cent were of *Fusarium*, whereas of 401 cultures made in October and November less than 9 per cent yielded *Fusarium*.

TABLE 5.—*Relative freedom of the Riverside tomato from nonparasitic defects and blemishes, as shown by fruit harvested in 1935 and 1936*

Variety	Year	Location of trials	Number of fruits inspected	Mean weight per fruit ounce	“Star,” cracked per cent	“Green-butts,” per cent	Sunburn per cent	Number of plants picked	Yield in pounds	
									First picking	Later picking
Riverside	1935	Riverside	457	4.4	18	14	13	10	21	83
Riverside	1935	Santa Ana	426	4.8		23				
Riverside	1936	Riverside	1732	4.4	9.0	21.6	4.1	60	34	593
Riverside	1936	San Onofre	68	5.8	27	34				
Marglobe	1935	Riverside	305	4.5	36	7	42	10	14	59
Marglobe	1935	Santa Ana	59	4.0		14				
Norton	1935	Riverside	465	4.4	51	3	18	10	19	94
Norton	1936	Riverside	1471	4.4	47.2	12.3	9.5	60	41	399
Stone selected	1936	San Onofre	68	6.1	39	38				

The Riverside tomato is not only highly resistant to the 2 wilt fungi, but it also shows a remarkable freedom from nonparasitic defects and blemishes.

The data in table 5 indicate that the Riverside tomato is less subject to radial, stem-end cracking (Fig. 1, B) and sunburn, but more subject to "greenbutts" or "greenback" than either Norton or Marglobe, 2 important varieties extensively grown in southern California. The cracking recorded in this table occurred during a period when no rain fell. With respect to its relative resistance to cracking, Riverside compares favorably with most of the varieties described by Boswell *et al.*⁷

Because of its firmness, relative freedom from radial stem-end cracking, and long-keeping quality, it is especially promising for long-distance shipping. A box of "mature green" fruit was shipped from Santa Ana, California, on October 13, 1936. On arrival at Hartford, Connecticut, 10 days later, 90 per cent of the fruits were ripe, 30 per cent were soft, no color defects, breakdown, or disease was found, and the condition of the fruit was satisfactory. Canning tests by the Laboratory of Fruit and Vegetable Products, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Los Angeles, and by the Arlington Canning Company, Riverside, California, gave satisfactory results. The outer wall seems to be unusually tenacious, so that when baked whole, the fruit maintains its shape and makes an attractive dish.

CONCLUSIONS

The Riverside tomato, developed by the U. S. Department of Agriculture and the University of California, cooperatively, was highly resistant to *Verticillium* and *Fusarium* wilts under the conditions of our experiments. When attacked by both fungi the *Fusarium* infection appeared to be much less frequent than the *Verticillium* infection.

In cultures made from stems of diseased plants, as a rule, *Fusarium* was predominant in the hotter part of the season, but *Verticillium* gained the ascendancy in the cooler weather of the fall. Not infrequently *Fusarium* was superseded by *Verticillium* in the same plant with the onset of cooler weather.

The Riverside tomato is suitable for both shipping and canning. It is adapted to localities with a long growing season. As a late shipping variety, it is especially promising because of its shape, firmness, late maturity, and relative freedom from radial stem-end cracks.

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RIVERSIDE, CALIFORNIA.

⁷ Boswell, V. R., *et al.* Descriptions of types of principal American varieties of tomatoes. U. S. Dept. of Agr. Misc. Publ. No. 160. 1933.

VERTICILLIUM WILT OF THE SUGAR BEET¹

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(Accepted for publication March 22, 1940)

INTRODUCTION

In August, 1939, an unusual disease of the sugar beet (*Beta vulgaris* L.) was found in the vicinity of Ault, Colorado. From root-tissue plantings on nutrient agar, a species of *Verticillium* was obtained, which was later shown to be capable of inducing the malady.

Although numerous economic crop plants have been reported as hosts of species of *Verticillium*, and more especially *V. albo-atrum*,^{3,4} reports of *Verticillium* attacking sugar beets are rare. Westerdijsk,⁵ in 1918, reported that she had observed a peculiar disease of sugar and forage beets for 10 years in The Netherlands, especially in the province of Zeeland and the island of Tholen. A characteristic symptom of the malady was the appearance of yellow spots on the outermost leaves of beets in late summer. The spots then sometimes spread over a large portion of the affected leaves, eventually bringing about the death of these organs. In severe cases, all leaves, with the exception of the youngest, were diseased. From the petioles and veins of such infected leaves, an isolate of *Verticillium* was obtained that, according to Miss Westerdijsk, could not be distinguished morphologically from cultures of *V. albo-atrum*. The pathogenicity of this organism to beet plants was demonstrated by planting seed that had been moistened with water containing the spores of the fungus. No statement was made regarding infection of the beet root.

Miles,⁶ in 1935, studied the virulence of strains of a *Verticillium* obtained from cotton in Mississippi, cotton in California, and Irish potatoes in St. Catherines, Canada. In regard to beets he wrote as follows: "Inoculations on eggplants, snapdragons, beets, and California poppy showed the Mississippi and California strains much more virulent than the fungus from Canada."

The literature reveals no report of the natural occurrence of *Verticillium* infection of sugar beets on this continent, hence, to the best of the writers'

¹ Contribution from the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Department of Botany and Plant Pathology, Colorado State College, Fort Collins, Colorado.

² Assistant Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture, and Assistant Pathologist, Department of Botany and Plant Pathology, Colorado State College, respectively. The writers wish to express their appreciation to Dr. G. H. Coons of the Division of Sugar Plant Investigations, U. S. Department of Agriculture, and to Dr. L. W. Durrell of the Department of Botany and Plant Pathology, Colorado State College, for advice and assistance.

³ Ludbrook, W. V. Pathogenicity and enviroanal studies on *Verticillium hadromycosis*. *Phytopath.* 23: 117-154. 1933.

⁴ Rudolph, B. A. *Verticillium hadromycosis*. *Hilgardia* 5: 197-360. 1931.

⁵ Westerdijsk, Johanna. Een *Verticillium* ziekte der suikerbieten. *Phytopathologisch Laboratorium "Willie Commelin Scholten"* Jaarverslag. 1917: 8-10. 1918.

⁶ Miles, L. E. The *Verticillium* wilt disease of cotton. (Abstract) *Phytopath.* 25: 972-973. 1935.

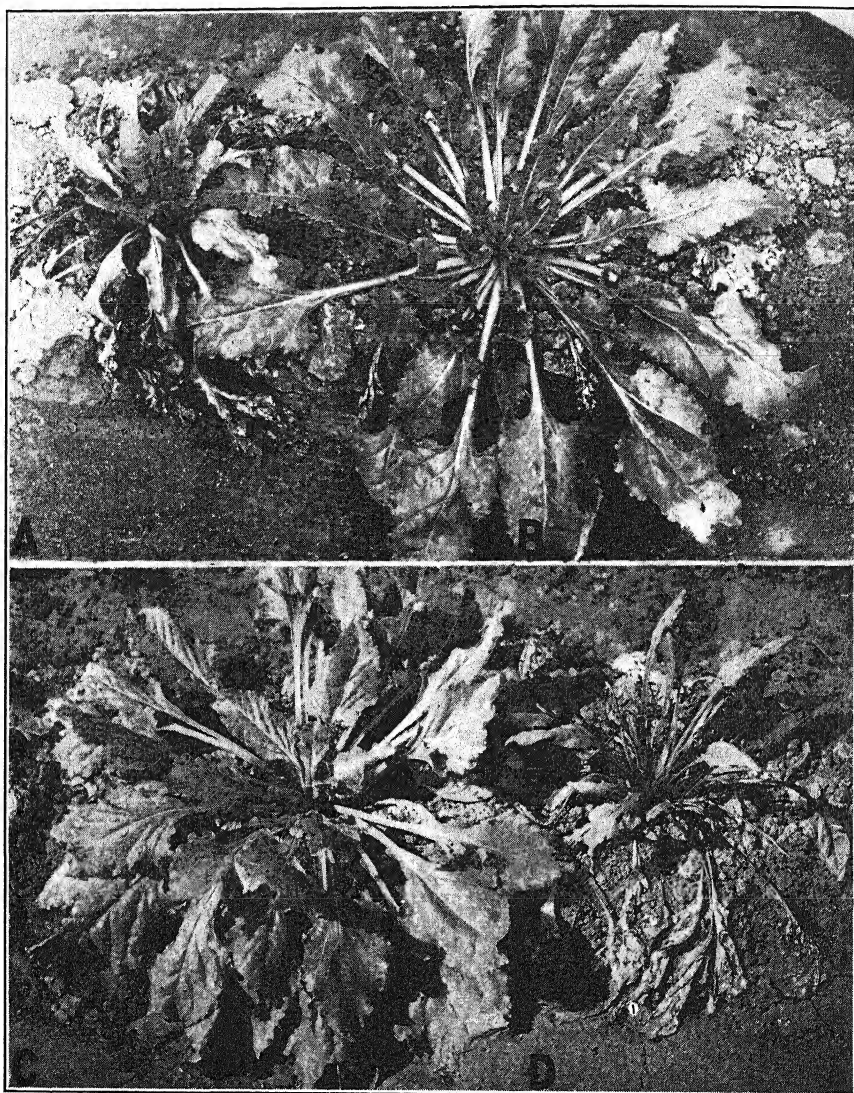


FIG. 1. *Verticillium* wilt of the sugar beet. A. Diseased plant showing an early stage of the wilt in which the only marked symptoms are wilting and death of the outer leaves. B and C. Healthy plants. D. Plant with an advanced stage of the malady. Note that, in the case of the affected plant, the outer leaves are wilting or dead, while the inner leaves are narrowed and pointed, tending to be twisted away from their normal positions, and showing a revolute curling of the margins of the blades.

knowledge this constitutes the first report of the occurrence of such a malady in North America.

THE DISEASE

In northern Colorado, attention was first drawn to affected plants because of the wilting and dying of the outer leaves (Fig. 1, A); the inner leaves of the plants being apparently normal, showing no loss in turgidity.

In later stages, as shown in figure 1, D, the inner leaves generally were narrowed, pointed, somewhat yellowed, and with a slight tendency toward becoming flaccid. The petioles of the inner leaves tended to be twisted away from their normal positions, and the leaf blades often showed a revolute curling of the margins.

The tap roots of diseased plants were solid and firm, with no evidence of rotting. On splitting the tap roots, however, it was found that a few of the vascular bundles were discolored, so that the general aspect was of white flesh, showing occasional brown or black strands. The tap root showed a relatively smaller number of these dark strands than did certain secondary roots. In many cases, it was possible to trace the necrotic vascular tissue from the tap root to a secondary root that probably was the path of invasion (Fig. 2).

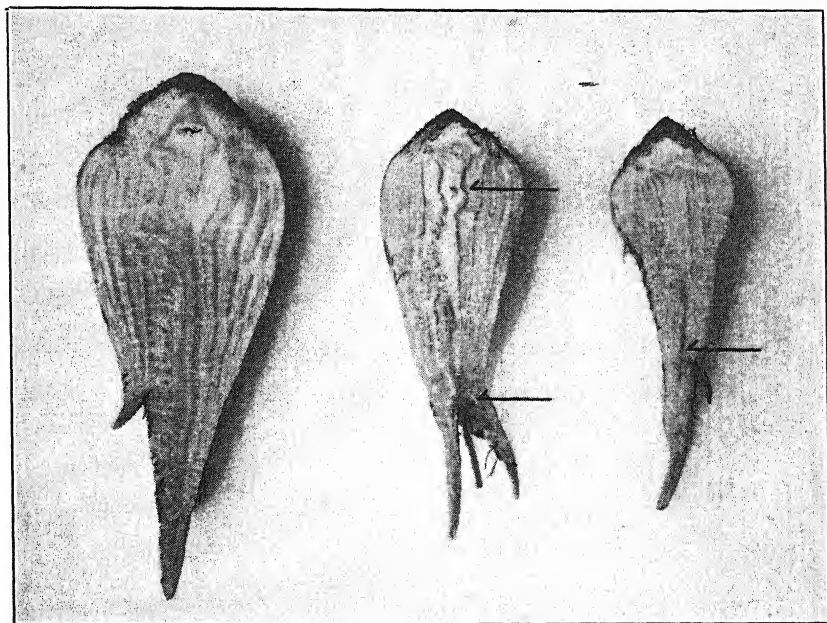


FIG. 2. Symptoms of *Verticillium* wilt in the roots of sugar beets. The beet on the left is unaffected, while the two on the right show *Verticillium* invasion. Certain necrotic vascular portions are indicated by arrows. Note in both affected beets that the pathogen apparently has made its entrance into the tap roots by way of the secondary roots.

THE CAUSAL AGENT

Tissue plantings on nutrient agar were made from roots of representative affected sugar-beet plants. From these plantings, pure cultures of a *Verticillium* were isolated. Because this fungus consistently appeared in the culture plates, it was tested for pathogenicity to sugar beets. This was done by introducing the fungus (agar-mat and barley giant-culture inocula) into previously steamed soil in pots, in which young sugar beets⁷ were trans-

⁷ Beets approximately three months old.

planted at the time the soil infestation was effected. Eighteen beets so treated, together with 12 noninoculated control plants, were placed in a warm greenhouse section, while a similar series was placed in a cool section of the house. During the 42-day interval in which the plants were exposed to infection, the warm section of the house was held at approximately 75° F., while the cool section was maintained near 60°. At the end of this interval, it was observed that 10 of the beets growing in *Verticillium*-infested soil in the warm section revealed characteristic symptoms of the disease (Fig. 3). No such indications were observed on comparable plants growing in the cool section. Plantings from the discolored vascular bundles of roots of wilting plants held in the warm section, yielded cultures that were morphologically identical with the isolates used to infest the soil. The control plants remained healthy, and tissue plantings from their roots did not yield fungous growth.

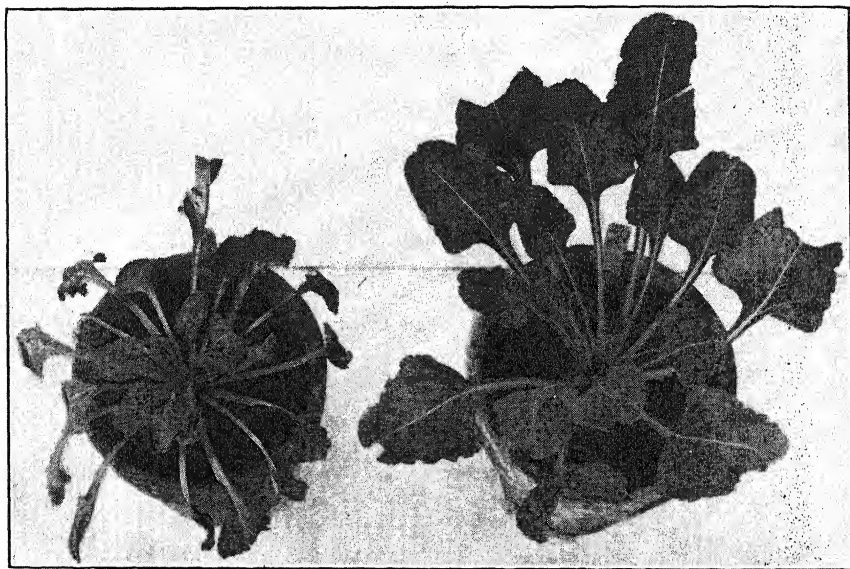


FIG. 3. The result of artificial inoculation with *Verticillium*. The wilted and stunted beet on the left is growing in steamed soil infested with *Verticillium*. A control plant of the same age growing in steamed noninfested soil is shown on the right.

The causal agent of *Verticillium* wilt resembled closely the descriptions of *Verticillium albo-atrum* Reinke and Berthold. The organism showed the characteristic verticillate conidiophores with conidia collected into heads on the sterigmata. Measurements of many of the single-cell, ellipsoid conidia of the organism, showed their sizes to vary from $3-10.5 \times 1.8-4.5 \mu$. Cultures of the fungus turned quite dark after a week's growth, producing dark hyphae and chains of dark mycelial chlamydospore-like cells.

THE PREVALENCE OF VERTICILLIUM WILT IN NORTHERN COLORADO

In order to obtain an indication of the prevalence of *Verticillium* wilt

of sugar beets in northern Colorado, a preliminary survey was made in 5 areas. A total of 11 representative fields was examined in the vicinities of Kersey, Lucerne, Ault, Fort Collins, and Loveland. Specimens showing apparent symptoms of *Verticillium* attack were taken from each field in which the presence of wilt was suspected, and tissue plantings on nutrient agar were made from all such roots for purposes of verification. As a result of this work the presence of *Verticillium* wilt was established in 7 of the 11 fields examined. In one field, near Ault, Colorado, representative counts were made of plants showing obvious foliage symptoms of *Verticillium* attack. These counts indicated approximately 1 per cent infected plants. The disease caused considerable damage in one end of this field, and an inspection of 100 consecutive plants in that area showed 10 plants with marked symptoms of the malady. In other fields in which the disease was found, the number of infected plants was considerably below 1 per cent. Symptoms of the malady in fields in the vicinities of Fort Collins and Loveland, in all but one instance, were either questionable or absent, and the roots of suspected sample plants taken from these fields yielded no cultures of *Verticillium*.

THE EFFECT OF THE MALADY ON THE SIZE AND QUALITY OF
SUGAR-BEET ROOTS

In order to obtain an estimate of the amount and type of damage caused by *Verticillium* infection in individual sugar beets, a total of 114 pairs of beet roots were taken at harvest time in accordance with an approved random-sampling procedure. These roots were obtained from a field in which the presence of *Verticillium* wilt had been verified repeatedly by isolations in the laboratory. Each pair included one plant that showed obvious foliage symptoms of *Verticillium* infection, and one control plant, which

TABLE 1.—Comparison of roots affected by *Verticillium* with adjacent apparently healthy roots. Results given as 5-sample averages of diseased and healthy roots. Each sample was made by compositing 20 or more roots of the respective class, taken at random

Samples	Total no. of roots	Aver. wt. per root	Aver. sucrose	Aver. coeff. of apparent purity	Aver. gross sucrose per root	Aver. ind. ^a avail. suc. per root
		lb.	%	%	lb.	lb.
Control roots	114	1.1488	15.86	92.52	0.18216	0.16846
Infected "	114	1.0584	11.50	88.58	0.12202	0.10798
Difference:						
Actual		0.0904	4.36	3.94	0.06014	0.06048
Per cent (based on controls) ...		7.87%	27.49%	4.26%	33.01%	35.90%
t		1.493	19.382	2.954	7.726	9.275
Odds ^b		<4: 1	>99: 1	>19: 1	>99: 1	>99: 1

^a An approximation, based on the assumption that a given quantity of impurities in sugar-beet juice will prevent the extraction of an equal quantity of sucrose. Actually, this tendency varies widely under different conditions.

^b Odds against the occurrence of the indicated difference being due to chance.

appeared to be noninfected. No roots were taken that showed evident rot; a condition seldom found. These beets were taken in 5 lots of approximately 23 pairs each, and the 2 types of roots in each lot were grouped to make a composite diseased sample and a composite control sample, respectively. Thus a total of 10 samples was obtained. All beets were topped at the lowest leaf scars, washed, weighed, and analyzed⁸ to determine sucrose percentages and apparent purity coefficients (Table 1).

The infected roots were below the controls in all phases studied, the differences being significant in all cases with the exception of root weight. In sucrose percentage, and in gross and indicated-available sucrose per root, the differences were highly significant.

The fact that the diseased roots were 27.49 per cent below the controls in sucrose percentage, and only 7.87 per cent lower in weight of root, was in keeping with the apparent late development of symptoms in the field.

SUMMARY

In the latter part of August, 1939, an unusual wilt of the sugar beet (*Beta vulgaris* L.) was observed in fields in the vicinity of Ault, Colorado. Isolations from the necrotic vascular tissues of the roots of affected plants yielded a species of *Verticillium*, which was capable of inducing symptoms characteristic of the disease. The morphology of the causal organism resembled closely the descriptions of *V. albo-atrum*.

Eleven fields were examined in five agricultural districts in northern Colorado, and *Verticillium* wilt was found in seven fields representing three districts.

In paired comparisons of diseased and apparently unaffected roots at harvest, the former class was found to be significantly lower in average percentage of sucrose, coefficient of apparent purity, and gross and indicated-available sucrose per root. The average weight of diseased roots was also lower than that of the controls, but the difference was not significant.

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A PRELIMINARY REPORT ON A FUNGUS DISEASE OF THE FIELD BINDWEED, *CONVOLVULUS ARVENSIS*¹

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INTRODUCTION

A fungus attacking field bindweed (*Convolvulus arvensis* L.) has been found at various locations in the Palouse area of Idaho and Washington, particularly on north slopes. Killed centers of bindweed spots were first

⁸ Bachler, Frederick R. The one solution method of analysis of sugar products. Facts about Sugar 28: 420-423. 1933.

¹ Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho Agricultural Experiment Station, as Research Paper No. 184.

noted by the writers in 1936. Since that time, they have been found at a number of points. A farmer near Genesee, Idaho, recalls definitely his having seen them in his fields as far back as 1931.

Isolations from diseased material were first made in September, 1937. Subsequent isolations have been made and used to inoculate seedling plants in the greenhouse, and typical infections have resulted. From these infections, 3 reisolations have been made. In culture the 3 reisolations appear to be identical with the fungus originally isolated. A brief description of the symptoms of the disease and of the causal fungus follows.

THE DISEASE

Field Symptoms

The field symptoms are characterized by infection first showing in or towards the center of a bindweed patch and thence spreading in all directions, more or less, with the spread of the bindweed. From this center of infection, plants become diseased and gradually die, so that eventually such centers may become devoid of bindweed plants and are surrounded by a ring of healthy, green plants, giving the entire patch a "doughnut" appearance (Fig. 1).

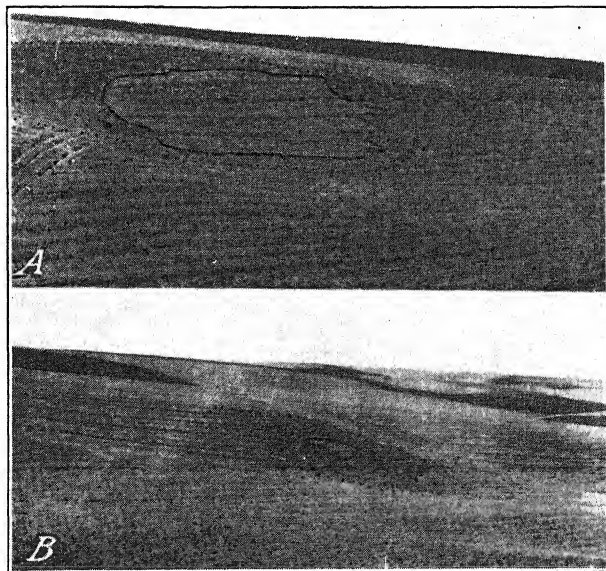


FIG. 1. "Doughnut"-shape spots of field bindweed. Areas inside the solid line, killed by the fungus; darker areas immediately outside, limits indicated by dotted line, were healthy bindweed. A. An old diseased spot with killed center approximately 62.5 x 91.5 ft. B. A young infection with killed center only approximately 10 ft. in diameter.

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³ Thanks are due Dr. George W. Fischer, Associate Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for assistance in the identification of the fungus organism.

The fungus-infested area shown in figure 1, A, was first observed by a farmer in 1931, who estimated that it was then approximately 10 ft. in diameter. It is on a steep, north slope of a hill, with one edge extending almost to the crest. The edge of the bindweed patch extends over the crest of the hill. Measurements of this spot were made in 1937 and again in 1939, with the results shown in table 1.

It will be seen that the principal direction of spread of the fatal infections was east and west along the sidehill rather than north and south. In the former direction the spread of the infection was more rapid than the spread of the bindweed, but in the latter direction the reverse was true. The reasons for these differences in direction and rate of spread are not yet definitely known. In most cases observed, however, the outlines, both of the bindweed patches and of the fungus-infested centers, were more nearly circular than was that shown in figure 1, A, with measurements given in table 1.

TABLE 1.—*Increase in diameter of a patch of field bindweed and that of the fungus-infested center devoid of bindweed plants*

Year	Diameter, in feet, of			
	Entire bindweed patch		Fungus-infested center	
	North-south	East-west	North-south	East-west
1937	100	151	61.0	74.0
1939	111	161	62.5	91.5
Increase	11	10	1.5	17.5

Adult Plant Symptoms

The symptoms on the individual diseased plants are characterized by the appearance of lesions at any point on the stem that may be in close proximity to the soil. At first the lesions are light-brown and have a rather watery appearance; later they become darker brown and hard. They range in size from small spots to 2 to 3 in. in length (Fig. 2, C). They may cover a portion or the entire circumference of the stem, causing death to all aerial parts above the point of infection (Fig. 2, B). After bindweed plants have been killed back repeatedly, the new shoots that may arise below the point of infection are very weak, with the leaves small, and appear as though they were suffering from depleted carbohydrate reserves in the roots. Likewise, the roots of infected plants are smaller in size, have fewer secondary roots, and in general appear to be suffering from malnutrition. The leaves may either turn yellow and die or, at the instance of infection, they may take on a water-soaked appearance and then die. Usually very little seed is set on the infected plants. In extreme cases the roots also may become infected; such infections, however, are much less common than on the stems and leaves. Pycnidia of the fungus have been found on old stem lesions in the late fall and early spring. No pycnidia have been found during the active growth of the plant.

Symptoms on Seedlings

Seedling infection has been observed only in the greenhouse on seedlings artificially inoculated or grown in soil infested naturally with the fungus. Under these conditions, the lesions are formed at the crown of the plant. At first they are light-brown, gradually becoming dark-brown to black and very hard. They appear first on one side of the stem and later gradually involve the whole stem (Fig. 3). The attacked plants eventually wilt and occasion-

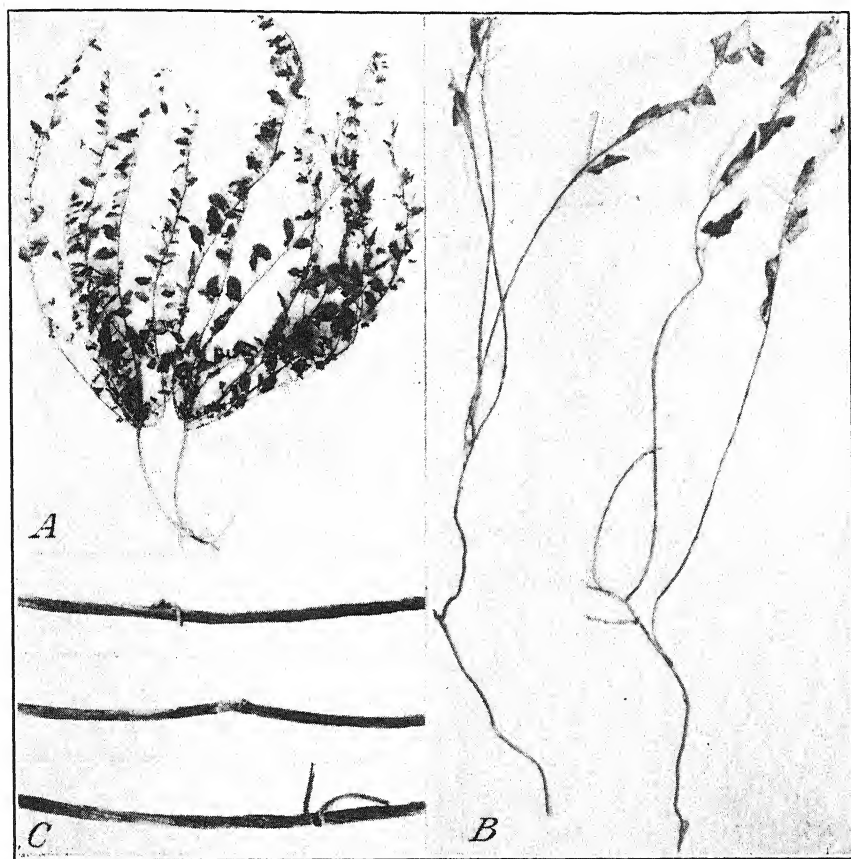


FIG. 2. A. Healthy field bindweed plant. B. Individual diseased plants, almost dead. C. Portions of diseased bindweed stems showing lesions.

ally the leaves turn yellow before the actual death of the plant. Infected plants are much lighter in color than noninfected ones.

THE FUNGUS

Pycnidia occur rather sparsely in the old lesions on the stems. They may be observed in the fall of the year after active growth on the plant has ceased. The pycnidia are rather firm-walled, formed of several layers of brown parenchymatous cells, which have thin walls. The pycnospores are

hyaline, filiform, straight or may be slightly curved, moderately attenuated at each end, pluriguttulate, 0-1 septate, $1 \times 35-37 \mu$. The fungus has been identified as *Rhabdospora* sp. The species has not yet been determined. To the knowledge of the writers, *Convolvulus* has never been reported previously as a host to *Rhabdospora*.

Limited culture work indicates that the fungus may be rather specialized in its requirements. Mycelial growth is very slow. Conidia are produced

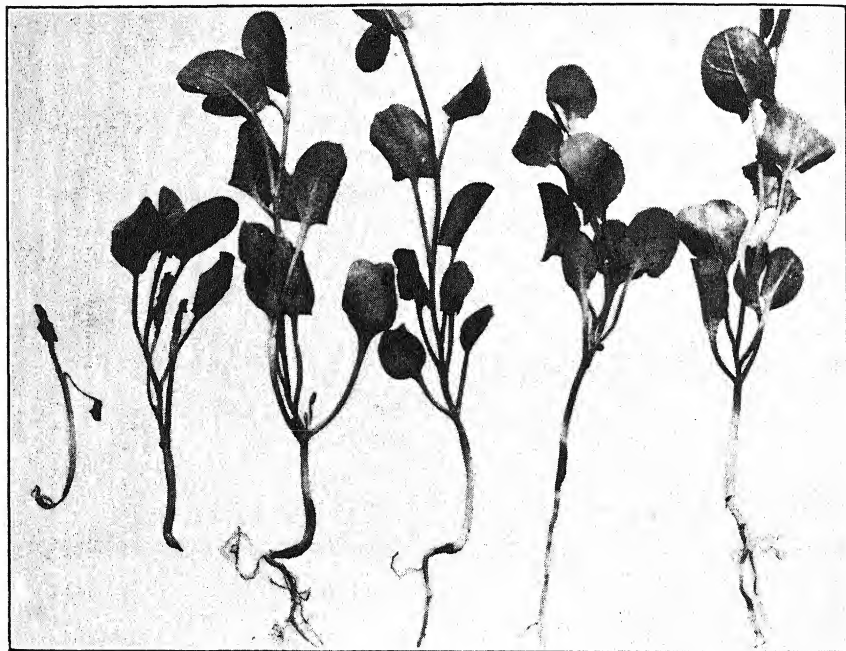


FIG. 3. Diseased and healthy field bindweed seedlings grown in the greenhouse: at left, 5 seedlings, artificially inoculated, showing characteristic lesions; at right, healthy seedling not inoculated.

in abundance when the culture is young. However, as the culture ages, conidial production stops entirely. Germination and subsequent growth are favored by moderate temperatures, about 20°C . No growth occurs in cultures held at 4° or 36°C .

A number of different methods of inoculation have been attempted. The most effective procedure was to place small mats of the fungus, grown in culture, on top of sterilized soil and then to plant the seeds on these mats and cover them with the same type of soil. By this method of inoculation, 95 to 100 per cent infection was secured. Lesions were apparent within 4 weeks, and by the end of 8 weeks the seedlings were all dead. Spraying a spore suspension on the leaves and stems failed to be entirely effective. Necrotic spots appeared on the leaves but no stem lesions appeared, nor was the growth of the plants impaired.

Field bindweed plants, started from seed, also were grown in the green-

house in soil naturally infested with the fungus. This soil was obtained from the fungus-infested area shown in figure 1, A. Thirty 8-inch pots were filled with this soil, 15 of them steam-sterilized and the other 15 left non-sterilized. Bindweed seeds were then planted in all of them and the pots placed in the greenhouse early in November, 1939. No infection occurred on the plants grown in the sterilized soil. In the nonsterilized soil 100 per cent infection was secured, the infection appearing first after 9 weeks. The symptoms were typical of those that occurred under field conditions. From these results, it is evident that the fungus is soil-borne.

Further studies of the disease are under way.

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A GREAT NORTHERN BEAN RESISTANT TO CURLY-TOP AND COMMON BEAN-MOSAIC VIRUSES¹

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(Accepted for publication March 16, 1940)

INTRODUCTION

Curly top and common bean mosaic are two of the most important bean diseases in Idaho and have caused severe losses to the bean-growing industry in Idaho. According to the mimeographed report of the U. S. Department of Agriculture (2), Idaho produced 928,000 hundred-pound bags of Great Northern beans in 1939, representing over 60 per cent of the Nation's crop of that variety. The total Idaho crop, of which the greater part is the Great Northern variety, represented about 11 per cent of the total bean crop of the United States.

The Idaho Agricultural Experiment Station has for a number of years been actively engaged in a bean-improvement program and has introduced Great Northern U. I. 123, U. I. 81, and U. I. 59 (4). These 3 selections now represent almost the entire Great Northern crop in Idaho. A brief review of other varieties introduced by the Idaho Agricultural Experiment Station and other agencies was reported in a previous paper (3).

Common bean mosaic has long been known to occur in beans. Reddick and Stewart (6) reported several varieties susceptible to the virus. Pierce (5) described a number of properties of yellow bean-mosaic virus (*bean virus 2*) and common bean-mosaic virus. A severe outbreak of curly top on beans was reported by Carsner (1), who first proved that the curly-top virus also would attack beans. Severe damage of garden-bean varieties and the variety Great Northern has occurred since that time in years when there are heavy infestations of the beet leaf hopper, *Eutettix tenellus* Baker.

¹ Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 185.

² The writer wishes to express his deep appreciation to Dr. C. W. Hungerford for his helpful suggestions throughout the course of this investigation and in the preparation of this manuscript.

Severin and Freitag (7) and many other workers have described the symptoms of curly top and properties of the curly-top virus.

This paper deals with the introduction to Idaho growers of Great Northern U. I. 15, which is resistant to the viruses of curly-top and common bean mosaic. The virus-resistant properties and some of the outstanding characteristics of the selection are discussed.

MATERIALS AND METHODS

Several experimental plots were established in southern Idaho to aid in the development of bean varieties resistant to disease-producing viruses. The plot near Buhl was located in an area usually severely infested with the beet leaf hopper. A wide variation in numbers of beet leaf hoppers and in the percentages of curly top found in the various susceptible selections has occurred at different seasons.

Although field selections in the Common Great Northern variety gave rise to selections resistant to common bean-mosaic virus, this was not the case when selections were made for resistance to the curly-top virus. Reciprocal crosses of Common Red Mexican, which is resistant to the curly-top virus and Great Northern U. I. 1, resistant to the common bean-mosaic viruses, were made by W. H. Pierce in 1929. From this material 2 Red Mexican selections U. I. 3 and U. I. 34, were developed (3). It is from this same hybrid material that several selections for beans of the Great Northern type, resistant to common bean-mosaic and curly-top viruses, were made.

For the past several years a program of continuous plant selection and testing for resistant Great Northern selections have been conducted. The writer made a number of selections in 1937 that included U. I. 15 and these selections have been compared with commercial Great Northern varieties. Great Northern U. I. 15 proved to be most valuable from several standpoints and has been increased as rapidly as possible on the trial grounds.

SYMPTOMS OF COMMON BEAN MOSAIC AND CURLY TOP

Common bean mosaic (*bean virus 1*) is caused by a seed-borne virus, transmitted in the field to susceptible plants by several species of aphids, and characterized by symptoms of severe leaf mottling and reduction in plant yield.

Field observations indicate that bean plants are more susceptible to the curly-top virus while in the younger stages of growth. Plants just emerged from the soil and those only in the primary-leaf stage are very susceptible, although field observations indicate that the plants may become infected during any stage of development. The first trifoliate leaf may curl downward, become thickened and brittle, and will easily break away from the stem (Fig. 1, B and C). Plants diseased at an early stage seldom recover or increase in size. The leaves become chlorotic and the plant usually dies in a very short time. Plants diseased late in the season may show curly-top symptoms at the top of the plant but may survive until the end of the season.

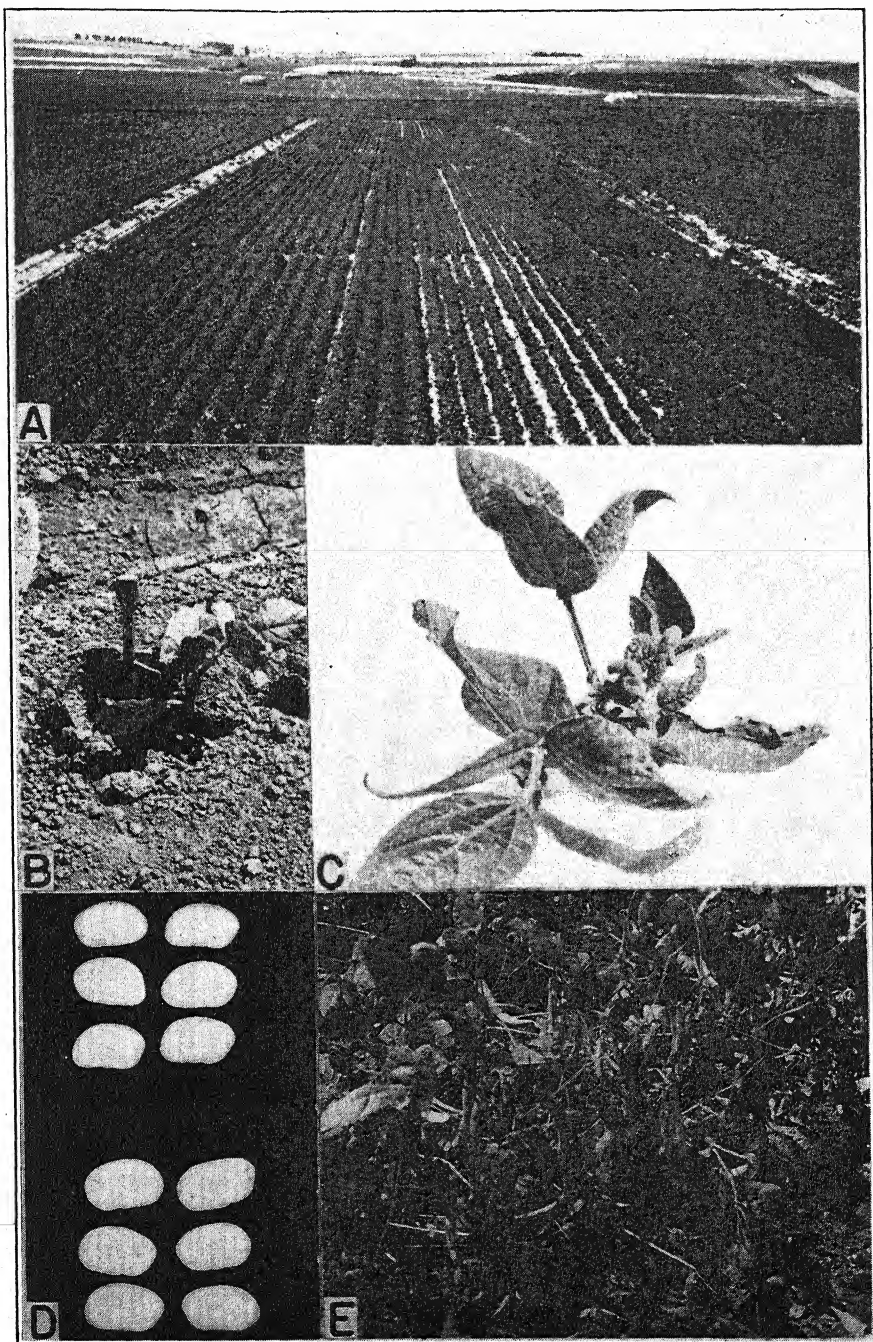


FIG. 1. A. General view of the Buhl experiment plot showing four-row plots of Great Northern selections in the foreground. B and C. Curly top diseased bean plants. Note in C the curled center leaves of the Red Kidney plant. D. Seeds of Great Northern U. I. 123 shown at the top and seeds of Great Northern U. I. 15 at the bottom. E. Mature plants of Great Northern U. I. 15 showing the set and shape of the pods.

Symptoms of curly top should not be confused with those of common bean mosaic or yellow bean mosaic after the initial stages of symptom expression. A slight chlorosis accompanied by a downward curling of the first primary leaf may be the first symptom of common bean mosaic. At this stage, a plant affected with curly top will show the primary leaf thickened, severely curled, and very brittle. Plants will continue to grow when affected with common bean mosaic and develop the typical mosaic mottling of the leaves; but, if affected by the curly-top virus, they cease growing, become chlorotic, and die; no mottling of the mosaic type will develop. The first symptoms of yellow bean mosaic are very similar to curly top, especially as to the downward curling of the first trifoliate leaf. In the case of yellow bean mosaic the light yellow spots will develop on the first trifoliate leaf and later will spread over the surface. As the plant grows, the yellow spots develop on the new leaves and thus differentiate this disease from both common bean mosaic and curly top.

YIELD AND QUALITY OF RESISTANT SELECTION

Each season during the program for the development of a Great Northern selection resistant to the curly-top virus, an effort was made to select those plants giving the most promise of having the desirable characteristics of that variety. Table 1 presents the yield data of Great Northern U. I. 15 together

TABLE 1.—Yield in pounds^a of 4 Great Northern bean selections grown on the experimental plot near Buhl, Idaho

Great Northern selection	1937		1938		1939				Average yield
	Plot I	Plot II	Plot I	Plot II	Plot I	Plot II	Plot III	Plot IV	
U. I. 15	31.3	34.6	25.9	19.5	24.6	24.3	26.7
U. I. 123	2.9	6.2	29.5	29.0	22.0	19.8	19.6	24.1	19.1
U. I. 81	5.0	7.9	28.0	28.6	22.1	25.0	23.7	19.5	20.0
U. I. 59	5.2	3.4	29.0	19.2	17.5	22.3	21.4	25.5	17.9

^a Plot yields taken from 50 feet of 4 rows.

with those of commercial Great Northern U. I. 123, U. I. 81, and U. I. 59. Data of 3 years' plantings on 2 to 4 plots each year are reported, except for U. I. 15, during the 1937 season, when seed was not available for plantings of this size. The table shows that the yields of the selections are very similar in those seasons when curly top was not severe (Table 2). However, in 1937, when curly top was very severe, the yields of the commercial selections were greatly reduced. Great Northern U. I. 15 was entirely free from curly top in 1937.

The leaf and vine characteristics of Great Northern U. I. 15 are, in general, similar to Great Northern U. I. 123. The foliage, however, is darker green. The runners are of average length in both selections with U. I. 15, giving somewhat more vigorous vine growth than U. I. 123. The seed size, shape,

TABLE 2.—Percentage of curly-top plants found in 4 Great Northern selections grown on the experimental plot near Buhl, Idaho

Great Northern selection	1937		1938		1939				Average
	Plot I	Plot II	Plot I	Plot II	Plot I	Plot II	Plot III	Plot IV	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
U. I. 15	0	0	0	0	0	0	0	0
U. I. 123	86.2	69.5	10.2	7.9	0.4	0.4	0.8	0.3	22.0
U. I. 81	87.1	53.9	18.0	13.6	0	0	0.5	0	21.6
U. I. 59	91.2	89.7	29.7	21.7	1.3	1.6	1.4	0.5	29.6

and color of Great Northern U. I. 15 are very similar to those seed characteristics of U. I. 123 (Fig. 1, D).

Cooking tests conducted by the Department of Home Economics, University of Idaho, show that dry beans gave no significant differences among the various selections. Great Northern U. I. 15, compared with other selections, was found of equal quality.

Soaking tests with a limited number of samples of the 4 selections show that U. I. 15 has a smaller percentage of beans with hard seed coats than do the commercial varieties.

On the Buhl plot 4 replications of Great Northern U. I. 15 were grown together with 3 commercial Great Northern selections. An average number of 92 days to reach maturity was found for U. I. 123, U. I. 81, and U. I. 59 and an average number of 95 days for Great Northern U. I. 15. Observations for other plots and other seasons indicate that the nature of the season and the fertility of the soil greatly influence the time of maturity of selections. Beans grown on very fertile soil tend toward later maturity. The difference of 2 or 3 days in maturity was found to be consistent when other plots were considered.

In table 2 are presented the readings of curly top found in the 3 commercial selections and Great Northern U. I. 15. No curly top or common bean mosaic has been observed in Great Northern U. I. 15 since it was first selected. Common bean mosaic was formerly very severe in Common Great Northern beans. The recently developed Great Northern beans, although resistant to the common bean-mosaic virus, are susceptible to the curly-top virus. Tables 1 and 2 show that in years of heavy infestations of the beet leaf hopper severe damage will occur in commercial Great Northern selections, especially in areas near the breeding grounds of the insect.

Great Northern U. I. 15, its parent varieties, and the commercial Great Northern selections mentioned in this paper, are all susceptible to the yellow bean mosaic virus (*bean virus 2*). The yellow bean-mosaic virus is not seed-borne and does not cause a serious bean disease in Idaho at the present time.

NAME OF THE RESISTANT SELECTION

The hybrid selection resistant to common bean mosaic and curly top has been named and numbered Great Northern U. I. 15.

The new selection will be grown on a few farms in 1940 in areas where the beet leaf hoppers are prevalent. The seed fields will be certified in the regular manner in order to develop for the growers a supply of seed free from mixtures of other varieties. Seed will not be available in large amounts until the fall of 1940. Great Northern U. I. 15 will be specially valuable to growers when planted in areas adjacent to the breeding areas of the beet leaf hopper where severe losses to Great Northern beans has occurred.

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REPORT OF THE 1940 ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twenty-fourth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of Washington, Seattle, Washington, from June 19 to 22, 1940, in conjunction with the summer meeting of the American Association for the Advancement of Science. Seventy-seven persons signed the register and 37 papers were presented during 6 half-day sessions. On Thursday morning statistical methods were discussed at a joint meeting with the American Association of Economic Entomologists, the American Society for Horticultural Science, and the Northwest Association of Horticulturists, Entomologists and Plant Pathologists. Friday morning was devoted to a symposium on virus diseases of fruit trees. On Saturday about 40 participated in a field trip under the direction of Glenn A. Huber and Karl Baur, and an opportunity was offered to observe several diseases of vegetables, small fruits, and tree fruits at the Western Washington Experiment Station, Puyallup, and vicinity. Several types of dusters and sprayers were demonstrated on the Station grounds.

Officers for the ensuing year are as follows: President, T. E. Rawlins, University of California, Berkeley; Vice-President, R. B. Streets, University of Arizona, Tucson; Secretary-Treasurer: C. E. Yarwood, University of California, Berkeley; Councilor, N. J. Giddings, U. S. Department of Agriculture, Riverside, California.

The next annual meeting will be held at the California Institute of Technology, Pasadena, California, in June, 1941.

Titles and abstracts of papers presented at the meeting follow.

C. E. YARWOOD,
Secretary-Treasurer.

Greenhouse Experiments for Control of Seedling Diseases of Sugar Beets. M. M. AFANASIEV and H. E. MORRIS. Soil and seed treatments with sugar beets were conducted to study their effect on the development of seedling diseases (black root) of beets. For soil treatment hydrated lime, sulphuric acid, formaldehyde, steam, and different combinations of nitrates, phosphates, and manure were used; and for seed treatments, basic copper sulphate, Ceresan, New Improved Ceresan, and a check. Four flats were used for each soil treatment. Each was planted with 4 randomized rows of treated beet seeds. Two plant-

ings of beets, with a growth period lasting a month each time, were made. Soil treatments were of great importance in controlling seedling diseases of beets, but seed treatments were slightly significant. Soil treated with nitrates, phosphates, and manure, and soils, sterilized with steam and disinfected with formaldehyde had the smallest amount of disease. The amount of disease present in different soils of the first planting showed that there was a definite negative correlation between the productive power of the soil and seedling diseases. Beets planted in each soil a second time had considerably more seedling diseases than those of the first planting, probably because of a decrease in the amount of nutrients in the soil and an accumulation of diseases.

The Use of Calcium Cyanamid and other Fertilizer Materials and Soil Amendments in the Destruction of Apothecia of Sclerotinia fruticola with Methods of Application. KARL BAUER and GLENN A. HUBER. Aqueous solutions of ammonium sulphate, sodium nitrate, and urea (fertilizer grade), although causing severe injury to apothecia of *Sclerotinia fruticola*, did not prevent subsequent development of these fruiting structures. Ammonium sulphate, hydrated lime, urea, and calcium carbonate, when applied just previous to the emergence of apothecia, did not provide an effective control. Calcium cyanamid alone effectively prevented the emergence of the apothecia, and, in addition, materially reduced the emergence of *Taeniothrips inconsequens*. Since commercial dusters were not suitable for application of calcium cyanamid, a machine with a capacity of 3 acres per hour was constructed. A "tapered boom" was found to provide a more even distribution of the material than the multiple "vegetable outlets" commonly used on dusters. A bur-lap hood was placed over the boom to prevent excess blowing of the calcium cyanamid.

A Canker and Die-back Disease of Overwintered Youngberry and Boysenberry Canes. EARLE C. BLODGETT. A serious cane injury on youngberry and boysenberry plants has occurred generally in Idaho in 1939, 1940. Red lesions, which usually first appear on the canes in the early spring about the time the plants trained on supports, later turn brown with a light colored center. Frequently, canes are entirely girdled and die back. Reduction of vitality of affected plants and loss of fruiting wood constitute the serious results of the disease. On infected canes, thornless youngberry in particular, fruiting structures of a fungus are present. Isolations from both cane cankers and spotted foliage have consistently yielded a fungus that agrees in general concept with *Septoria rubi*. Inoculation tests with pure cultures have produced typical cane and leaf symptoms in about 3 weeks after inoculation in the greenhouse. Spraying tests with Bordeaux mixture and lime sulphur in October, 1939, gave no apparent reduction in cane lesions in 1940. *Septoria rubi*, although occurring generally, usually produces a leaf spot instead of a cane canker and dieback, as reported herein. Tests with protective shading of the canes during the winter indicate that the trouble is not caused by winter injury or sun scald, although these factors may in some years contribute greatly to the extent of the damage.

The Strip Method of Soil Treatment for the Control of Black Root of Sugar Beets. LEO CAMPBELL. Seed treatment with two per cent Ceresan has consistently resulted in degrees of control of only the early preemergent stage of black root of sugar beets, but sufficient to give up to 25 per cent increase in unthinned stands. In attempts to control the late preemergent and the postemergent stage of the disease, soil from strips 3 in. wide and 1½ in. deep was removed and thoroughly mixed in a bucket with fungicides to be tested, replaced in the original strip in the field and nontreated seed drilled therein. Pulverized and oiled calcium cyanamide added at the rate of 135 lb. per acre a week before planting resulted in an average decrease in percentage black root of 53.6, an average of 231 per cent increase in healthy plants and gave an excellent growth of annual beets. Formaldehyde dust, New Improved Ceresan, and hydrated lime, added in the proper amounts at planting time, were only slightly less effective in the control of black root. Equipping of a beet drill with attachments for thoroughly mixing the fungicides in the soil is being planned.

Occurrence of Big Bud of Tomato in the Pacific Northwest. B. F. DANA. Big bud of tomato, a disease believed to be new to the North American continent, appeared very sparingly in the Pacific Northwest in 1937, 1938, and 1939. Marglobe, Dwarf Champion, and Bonny Best varieties and miscellaneous lots of *Lycopersicon* spp. from South America were affected. Branches were increased in number and clumped into witches' brooms. Abnormal inflorescences with increased subdivisions and phyllod flowers were borne on these clustered branches. Phloem proliferation was constantly associated with the abnormal inflorescences and branches. The disease was successfully transferred from tomato to tomato by grafting buds from diseased into healthy plants with an incubation period of a month. Juice transfers were not successful. Phyllody accompanied by increase and clumping of branches also appeared on common bean, Lima bean, soy bean, alfalfa, sweet clover, squash, and carrot. Simultaneous occurrence and similar appearance of phyllody on these hosts suggest a possible relationship to big bud of tomato.

Resistance and Susceptibility to Curly Top in Varieties of Common Bean, Phaseolus vulgaris. B. F. DANA. Extensive field trials have shown extreme susceptibility to curly top in varieties of wax-pod snap beans, moderate to extreme susceptibility in varieties of green-pod snap beans, and extreme susceptibility to strong resistance or immunity in varieties of field or dry beans. Strong resistance was found only in California Pink, California Red, Red Mexican, Burtner, and Jenkins or Idaho Cream. Curly top of beans may kill plants of susceptible varieties in the seedling stage. Older plants, severely diseased, may gradually turn yellow and die. The growing points of susceptible seedlings and older plants usually are killed early and may fall away. Older plants continuing to grow after infection are dwarfed, internodes are shortened, leaves are reduced in size and curled, often forming a mass of curled foliage. A large number of varieties of susceptible snap beans and a few susceptible field varieties were crossed with resistant Burtner, Red Mexican, and California Pink, for production of snap bean varieties resistant to curly top. Progeny selections in the fourth generation of Blue Lake \times Burtner and the reciprocal cross exhibited strong resistance and excellent snap bean quality. Additional resistant green-pod and wax-pod strains may be developed from other hybrid progenies now available.

A Preliminary Report on the Inheritance of Resistance to Rust (Uromyces appendiculatus) in Beans (Phaseolus vulgaris). B. DUNDAS. The inheritance of resistance to 4 physiologic strains of rust in beans has been studied by inoculating detached leaflets of plants from F_2 populations floated bottom side up on a 5 per cent sucrose solution in Petri dishes. By this method the same plants can be tested for their reaction to a number of strains. Rust strains used were 1 and 2 from Dr. L. L. Harter, and two others isolated from material from Florida and Washington and tentatively identified by Dr. Harter as his strains 10 and 4. Test of F_2 plants of Brown Kentucky Wonder 36928 \times susceptible Pinto indicate that 36928 contains three main independent dominant factors for resistance, A, B, and C. The factor A, alone or in combination, gives resistance to strains 1, 2, or 10, B gives resistance to strains 1 and 2, and C resistance to 10. The variety carries no factor for resistance to strain 4. Test of F_2 plants from the cross of the two resistant varieties 36928 \times Golden Gate Wax indicate that Golden Gate Wax contains a single dominant factor for resistance to strains 1, 4, and 10, which is different from three factors in 36928 and is designated as D. This factor gives no resistance to strain 2. Other data indicate that still different factors for resistance are present in other varieties.

A New Factor for Resistance to Powdery Mildew (Erysiphe polygoni) in Beans (Phaseolus vulgaris). B. DUNDAS. In earlier studies a single dominant factor for resistance to mildew was found in Pinto and other field-bean varieties. The same factor also has now been found in one variety of snap beans, a strain of Kentucky Wonder. A number of bean varieties have been observed to be only slightly infected in the field, but, when subjected to a dish-test, gave a more susceptible reaction. The varieties do not contain the Pinto factor and can be classed as semiresistant, their reaction to mildew varies considerably from year to year, depending partly on the strains of mildew present. F_2 plants of the cross of the semiresistant Long Roman \times the susceptible Red Kidney were inoculated in the field at an early date with strain 1 of the mildew. Ninety-four plants were semiresistant, like the Long Roman, and 32 were susceptible. This ratio of 3:1 indicates that the resistance in Long Roman is due to a main dominant factor. F_3 families from susceptible F_2 plants gave only susceptible populations. Of the F_3 families from semiresistant F_2 plants, 29 were homozygous semiresistant and 52 segregated in a ratio of 3 semiresistant:1 susceptible, confirming the F_2 results that the semiresistance in Long Roman is due to a single main dominant factor.

Curly-top Virus Strains. N. J. GIDDINGS. Infection of beets of a susceptible variety by the less virulent curly-top virus strains 2 or 4 does not alter the susceptibility of those plants to the more virulent strains 1 or 3. Plants infected by virulent strain 3 may be subsequently infected by the less virulent strain 2. Plants infected by strain 3 are quite resistant to infection by strain 1. Susceptible beets infected with strains 2 and 3 normally develop severe symptoms; but, passing this virus mixture from them through a resistant beet, variety 68, and back to susceptible beets, usually results in an expression of mild symptoms. This might be considered due to attenuation, but actually it is evidence of reisolation of strain 2. If leaf hoppers, fed for a few hours on each of two or more virus strains, are daily transferred to new test plants, the same leaf hopper may infect some plants with one virus strain, some with another, and some with combinations of two or more strains. Sometimes the virulent strain 3 is masked in an infected susceptible beet, showing only the mild, strain-2 symptoms. Subsequent transfers from such plants cause some plants to be mildly diseased and others severely diseased. Such results might be interpreted as due to restoration of virulence, but actually it is evidence of reisolation of strain 3.

The Origin and Inheritance of M Types in Hypomyces. H. N. HANSEN and WILLIAM C. SNYDER. During the past several years, while critically studying numerous isolates of many species of *Fusarium*, it was found by single-spore analysis that isolates of a given species existed in nature in the homocaryotic mycelial (M) form, the homocaryotic conidial (C) form, or in the heterocaryotic MC condition. M types also have been repeatedly observed to arise *de novo* in pure C-type cultures of various species. So far, C types have not been observed to arise in pure M-type cultures, indicating a directional change away from the C type under certain conditions. Among the *Fusaria* behaving in this manner were several isolates of a parasitic form of *F. javanicum* Koord. (*Hypomyces ipomoea* Halst.), a heterothallic fungus most often obtained from nature in the conidial form, which produces microconidia on sporodochia, perithecial fundaments, and relatively little aerial mycelium. The M types produce microconidia, relatively few macroconidia, no sporodochia, no primordia, and abundant aerial mycelium. When M- is crossed with C+, analyses of the resultant ascospores reveal that characters M and C segregate in the normal Mendelian ratio of 1:1 and independently of sex reaction. Other variants arising in culture and ecological variants obtained from nature inherit in the same manner, showing that variation in this fungus is genetic rather than cyclogenetic, and suggesting that this may hold true for fungi in general.

California Regulatory Laws and Fruit-tree Viroses. J. LEE HEWITT. Fruit-tree viroses that are dangerous or detrimental to agriculture are defined by law to be "pests" and, as such, are subject to regulatory action (1) by State-attempted eradication (in the case of peach mosaic disease this is cooperative with the U. S. D. A.); (2) by formal intrastate plant quarantine established by the Director of Agriculture and administered under his direction by county agricultural commissioners; (3) by rejection at destination inspection because of the discernible presence of the disease; (4) by rejection at destination for reasonable cause to presume the liability of infection; (5) by Hold Order, refusing permission to remove infected material from the premises where found. In all such regulatory action popular support of the action is essential. When "reasonable cause to presume" is invoked, added desiderata are: a known supply of clean material, adequate explanatory publicity, and freedom from infection at the destination, whether it be a nursery or a larger area. Reasonable cause to presume ceases to exist when there is adequate certification of treatment or of inspection and cleanness. The registry of citrus trees inspected for psorosis provides a suitable known supply of clean material upon which regulatory action could be based when it becomes desirable.

Net Necrosis of Potato in Western Washington. GLENN A. HUBER. Inspections of commercially grown potato tubers in Snohomish and Skagit counties in the fall of 1938 showed that from a trace to 30 per cent were affected with strong net necrosis. Inspections of the 1939 crop showed up to 42 per cent of the tubers affected. The necrosis often extended throughout the flesh of the tubers and the affected tubers usually produced spindle sprouts. An examination of 201 hill units of certified Netted Gem, selected for seed stock in 1938, revealed that 84 units or 41.8 per cent, included one to several necrotic tubers. These tubers were planted in the field in the spring of 1939, each unit being planted separately. Tubers showing necrosis produced vines that developed symptoms similar, although different in some respects, to leaf roll, while tubers from the same hills showing no necrosis produced apparently healthy vines. First symptoms of current infection on vines were similar to those caused by *Rhizoctonia* (*Corticium vagum*) infection with the exception of aerial tuber formation. A part or all of the vines in a hill may become infected. Only those vines showing symptoms of the disease produced necrotic tubers.

The Stony-pit Virus of Pears. J. R. KIENHOLZ. Symptoms of this relatively new disease were illustrated by means of fresh samples of fruit, leaves, and twigs. The most noticeable symptom occurs on the fruit in the form of pitting and deformity. A leaf symptom consisting of a veinlet chlorosis has been found associated with pitted fruits; but this occurs only on selected leaves and often becomes masked as the season progresses. Pear varieties differ greatly in their susceptibility to stony pit and in the symptoms expressed. Recent work has been devoted to a technique to make use of leaf symptoms in identifying the disease on nursery trees. Of the varieties tested, The Forelle, French seedling, and Bosc have yielded the most consistent leaf symptoms. Different symptom expressions within the same variety, however, have raised perplexing questions. The appearance of veinlet chlorosis in supposedly clean stock also suggests that the virus may be quite generally present in some varieties in a latent form. An indication that passage through one variety may attenuate the virus toward another also has been observed. Masking of symptoms, due to unknown causes, has complicated the standardization of the leaf test. Only Bartlett and Comice, of the common western commercial varieties, have produced sound fruit from infected trees.

Influence of the Pathogen, Environment, and Host Response on the Efficacy of Seed Treatment with Sugar Beets and Some Vegetable Crops. L. D. LEACH. Damping off of sugar beets, spinach, cowpeas, or watermelons caused by *Pythium ultimum* was satisfactorily controlled by seed treatment with red oxide of copper, but organic mercury compounds were more effective when the infection was due to *Rhizoctonia solani*. The 3 pathogens most frequently responsible for damping off of sugar beets in California are *Pythium ultimum*, *Rhizoctonia solani*, and *Phoma betae*. The first two are common in field soils, while *Phoma* appears to originate only from imported beet seed. Of 52 lots of European sugar-beet seed examined by microscopic examination of incubated seeds or by laboratory isolation, more than half showed some *Phoma* infestation, and 8 showed more than 10 per cent of the seed balls infested. None of the 19 lots of domestic seed examined by the same methods showed *Phoma* infestation. Infested seed lots showed *Phoma* damping off when planted in steamed soil. Satisfactory control was secured by treating the seed with any of the organic mercury compounds tried, but not by the use of copper or zinc compounds. Sugar-beet seedlings in soils of pH 5.0 or lower often exhibit acid injury that in some ways resembles damping off but that can be controlled by liming. A late form of black root differing from true damping off and apparently not controllable by ordinary seed treatments was serious in some peat soils in central California in 1940.

Separation of Tulip 1 Virus from Lily-latent by Cytological Methods. F. P. McWHORTER. The term viroplast has been proposed as a general term for cytoplasmic masses or "bodies" associated with, or resulting from, the presence of a virus within a cell. Viroplasts differ greatly in composition and structure, but the differences are hard to demonstrate without exceptional optical equipment. A study of tulip material inoculated with lily and tulip virus has shown that Trypan Blue in physiological salt solution is remarkably effective for differentiating viroplasts produced by different viruses. Tulip 1 virus produces a homogenous type that cannot be resolved into a particulate or reticulate structure with a 3650 Ångstrom lens system. This viroplast stains characteristically with Trypan Blue, a fact that has enabled us to demonstrate readily the absence of these in tulips infected with lily latent virus, although the latter produces abundant "x-bodies" in tulips and the external leaf symptoms are essentially the same as Tulip 1.

A Phytophthora Disease of Chamaecyparis. J. A. MILBRATH. A destructive root and crown rot of *Chamaecyparis* was first observed in 1938. The disease is now causing extensive losses in commercial and private ornamental plantings. The affected trees first show a general discoloration comparable to the progressive color changes that occur on the foliage of these trees when the roots are cut off or injured in such manner as to bring about sudden death to the tree. Eventually all the foliage changes to a reddish brown indicating the death of the tree. When the discoloration first appears on the foliage, the roots are already dead and the advancing edge of the fungus has extended into the crown up to the soil level. A sharp line of demarcation between dead and living tissue can be observed at this point by removing the outer layer of bark. The same species of *Phytophthora* has been isolated from a number of diseased trees. Plants inoculated with this fungus have died in 2 to 6 months and the same species of *Phytophthora* has been reisolated. Cultures of this fungus have been studied by C. M. Tucker of the University of Missouri, who has suggested the name *Phytophthora lateralis* for this apparently new fungus.

Camellia Yellow Spot—A Virus Disease. J. A. MILBRATH and F. P. McWHORTER. Nurserymen have observed that camellias, propagated from certain stock plants, consistently show such leaf abnormalities as yellowing, mottling, and epidermal roughening or corkiness. Neither bacteria nor fungi have been isolated from leaf tissue showing any of these symptoms, although the corkiness suggests a fungus organism. When portions of mottled plants were grafted to plants with normal foliage, the new leaves that formed above the point of union became mottled and yellow. Likewise, when portions of healthy plants were grafted to mottled stock, the foliage arising from the scion bud was affected. It would seem, therefore, that a virus is concerned. This concept may explain many of the foliar abnormalities commonly present on camellias. These leaf abnormalities include a mottle composed of individual, circular, cleared zones, yellowing or whitening of the leaves along the veins or along the edges, roughening or corkiness of the epidermis, and occasionally indications of advanced necrosis. A study of many varieties suggests that these symptoms are of similar origin. In order to facilitate the propagation of healthy stock it will be necessary to determine a method of distinguishing virus effects from natural variegations and albinism.

Further Studies on the Comparative Efficacy of Bordeaux Mixture and Some "Insoluble" Copper Sprays for the Control of Walnut Bacteriosis in Oregon. P. W. MILLER. In studies carried on in western Oregon in 1939, the following "insoluble" copper com-

pounds were tested under field conditions and their phytocidal effect and relative efficacy for the control of walnut bacteriosis determined: copper oxalate, yellow cuprous oxide, copper oxychloride, copper acetate, zinc ammoniacal copper silicate, brown cupric oxide, and a proprietary copper fungicide containing 34 per cent metallic copper. Of these, copper oxalate 3-100, yellow cuprous oxide 3-100, copper oxychloride 3-100 and copper acetate 3-100 gave good to excellent control of the disease without injury under the conditions prevailing in Oregon in 1939, comparing favorably with Bordeaux mixture in effectiveness.

Rusty-mottle, A New Virosis of Cherry. E. L. REEVES. A graft-transmissible disease of sweet cherries, referred to by the common name of "rusty-mottle," is described. In accordance with the system of virus nomenclature proposed by Holmes the binomial *Marmor rubiginosum* is suggested. There are no apparent early-spring symptoms on blossoms or leaves. The small basal leaves exhibit a chlorotic mottling from 4 to 5 weeks following full bloom, rapidly develop late-season colors of bright yellow to red with islands of green, and abscise. Thereafter, variable chlorotic mottling of all the foliage takes place, and many of the oldest leaves rapidly develop autumnal colors and subsequently drop. Leaf casting, affecting 30 to 70 per cent of the foliage, takes place largely during the 7th and 8th weeks following full bloom. The mottling of the remaining foliage then becomes more pronounced, the chlorotic spots and areas becoming yellowish-brown, resulting in a general rusty appearance of the foliage. Fruits on affected trees are smaller, retarded in maturity, insipid in flavor, but not misshapen. The growth and general vitality of an affected tree are gradually reduced. The disease has been repeatedly transmitted in the past 5 years by grafting or by some adaptation of the grafting process, but not by inoculation with expressed juice. The evidence indicates that it is caused by a virus.

A Sweet Clover Ring Spot from Alsike Clover (Trifolium hybridum). SAUL RICH. A virosis, occurring naturally on alsike clover near Amity, Oregon, was found to be readily transmissible by the carborundum method, to white sweet clover (*Melilotus alba*). The symptoms produced on white sweet clover under greenhouse conditions were local lesions and vein necrosis on the inoculated leaves, followed by systemic infection causing irregular chlorotic or necrotic lines, blotches, and ring spots. Severe infection resulted in leaf distortion, rosetting, and stunting. Symptoms very similar to these have been observed occurring naturally on sweet clover in Oregon. The known host range thus far includes 11 species of leguminous plants. Solanaceous hosts have not yet been demonstrated, although the virus has been inoculated into both *Nicotiana tabacum* and the tomato. The virus remains active after 5 days *in vitro*, but is inactivated by heating for 10 minutes at 55° C. Infected *Melilotus alba* plants produced symptomless foliage during a period of high greenhouse temperatures. These young symptomless leaves contained no transmissible virus, while the older, visibly affected leaves on the same plants contained transmissible virus.

An Apparently Undescribed Storage Rot of Grapefruit. R. B. STREETS. During April, 1939, an apparently undescribed fruit rot occurred in Arizona grapefruit held in cold storage. The only external symptoms were a blackening and loosening of the button and a slightly "brassy" color of the peel; but longitudinal sectioning revealed, in early stages, a browning of the vascular bundles. Intermediate stages, which developed within 7 to 14 days after removal of fruit from storage, showed a dark-brown rot involving several of the segments. In advanced stages the peel was also affected by a pliable, dark-brown rot which eventually enveloped the entire fruit. All stages were accompanied by a mild to decidedly unpleasant flavor pervading the entire fruit. No external sporulation was observed, but the discolored tissues within the fruit were filled with the dark mycelium and muriform spores of *Alternaria citri*. The difficulty of detecting and removing all infected fruit in the packing house, rather than the small percentage of fruit infected, makes the disease worthy of note. The end rot of navel orange was at least twice as prevalent in Arizona groves in the fall of 1939 as in average years, but the storage rot in grapefruit was less in 1940 than in 1939, due largely to care in selection of fruit promising a good cold-storage life.

Results of Three Years' Treatment of Pecan Groves for Control of Phymatotrichum Root Rot. R. B. STREETS and L. A. BRINKERHOFF. For 3 years (1937-1939) data were secured on the number of new infections and the number of pecan trees killed each year by *Phymatotrichum omnivorum* under 5 different conditions of intercropping and soil treatment. The plots were 80-acre orchards containing a total of about 6,800 trees. Of this number 1,636 trees, or 24 per cent, became infected; in the 4 best plots, in which infected trees were promptly treated, a total of 129 trees, or 1.5 per cent, died; while in the non-treated plot 133 trees, or 10.6 per cent, died. Likewise, the small number of new infections and trees killed from June to November, 1939, in plots, 1, 2, and 3 (5, 21, and 16 trees

infected, and 1, 3, and 3, trees killed, respectively) indicates that the disease is under control in these orchards. Most of the treatments were combinations of ammonium sulphate or ammonium phosphate and agricultural sulphur in dosages varying from a basic treatment of 1 lb. of each chemical to each 10 sq. ft. of root area followed by a 4-in. irrigation. The root area was treated to the drip of the branches. A 10-acre plot in a severely root-rot-infected but nontreated grove, which had an intercrop of alfalfa in 1937 and 1938, had 4.4 per cent dead trees in June, 1937. Three years later 29.5 per cent more trees were dead, and 37 per cent infected, leaving only 29 per cent apparently healthy trees.

The Increase of Tobacco-mosaic Virus and the Changes Accompanying Nitrogen Compounds in Detached Tobacco Leaves. WILLIAM N. TAKAHASHI. Detached mature tobacco leaves that had been inoculated with tobacco-mosaic virus 4 weeks prior to detachment (mosaic), healthy leaves inoculated with a 1:100 dilution of mosaic-leaf extract at the start of the experiment (mosaic inoculated), and healthy noninoculated leaves were cultured in distilled water in the dark. The distribution of Kjeldahl nitrogen in the supernatant liquid (soluble nitrogen) after treatment of expressed juice with trichloroacetic acid, the resulting precipitated material, and the leaf residue were followed during the culture period. One half of each sample was treated with trypsin in order to digest host proteins and the other half was maintained as a control. As the culture period progressed the control samples in all 3 cases showed a conspicuous increase in soluble nitrogen largely at the expense of the leaf-residue nitrogen. In the trypsin-treated samples an increase in the trypsin-resistant trichloroacetic precipitable fraction during the culture period was observed, even in the healthy leaves. The extract from the trypsin-treated samples was heated for 20 minutes at 70° C. and inoculated on *Nicotiana glutinosa* half leaves. Increasing numbers of local lesions as the experiment progressed showed that virus increase had taken place in mosaic leaves and particularly in the mosaic-inoculated leaves. Virus increase took place in mature leaves in the absence of photosynthesis, additional external mineral or organic nutrients, and in the face of accelerated katabolism.

Graft Transmission, Persistence and Migration of Some Viruses in Fruit Trees. H. EARL THOMAS. Winters peach-mosaic virus was transmitted from naturally infected almond by using old infected spurs as inoculum. This virus may move rather rapidly in either direction, producing symptoms 8 inches from point of inoculation in 19 days, or very slowly, as in *Prunus andersoni*, in which distal movement is greater. Defoliation accelerates movement into peach branches. The virus may persist for months in sweet cherry without any characteristic symptoms and in *Prunus lusitanica* without any symptom at all. The leaf-casting (buckskin) virus of cherry and peach seems to be more readily transmitted by grafting from sweet cherry to peach than from peach to cherry or peach to peach. Leaf symptoms develop typically in relatively mature leaves, but seem dependent on the presence of virus during growth. A group of diseases of apple, apricot, cherry, pear, plum, and prune characterized by roughening of bark or distortion of wood, and mostly of uncertain etiology, seem to have in common a very slow rate of development and scarcity of symptoms in other parts of the tree. Some of these persist in scions and some appear to be infectious.

Tulip Anthracnose. C. M. TOMPKINS and H. N. HANSEN. An anthracnose disease, affecting the peduncles and leaf blades of Darwin tulips (*Tulipa gesneriana* var. *darwinia*) occurs at Burlingame, California. Symptoms of the disease consist of small to large elliptical lesions parallel to the long axis of the peduncle. At first, the lesion is water-soaked in appearance, but later it becomes dry with a black margin, and numerous fruiting bodies of the fungus develop in the central area. The causal fungus was readily isolated by tissue plantings or by spore transfers to potato-dextrose agar. Young tulip plants were inoculated by atomizing with a water suspension of spores, and infection was obtained in 7 to 10 days. Reisolates of the fungus also proved pathogenic. The fungus has been identified as *Gloeosporium thumentii* f. *tulipae*, forma nov. In nature, the varieties Reverend Eubank and Zwanzburg are highly susceptible, while Clara Butt and Fantasy (parrot type) appear to be immune. The means of overwintering of the fungus has not been ascertained.

Low Germination of Peas Associated with the Presence of Bacteria in the Seed. W. J. VIRGIN. Seed peas of the large-seed, wrinkled varieties often germinate very poorly. One of the primary causes of low germination appears to be traceable to bacteria in the seed. A large number of isolations were made from different lots of seed that were first sterilized in a solution consisting of 500 cc. of 70 per cent alcohol and 1 g. HgCl₂ for 5 to 20 minutes. Invariably, bacteria were obtained from the seed of some of the lots. These bacterial isolates varied in their pathogenicity in regard to reducing germination. Seeds were inoculated by soaking the seed in nutrient broth in which the bacteria were growing. In some of the tests the seed coats were chipped so the bacteria could get under the seed coat. The

germination of seeds with unbroken seed coats was not affected by soaking in the bacterial broth, but the germination of those with chipped seed coats was considerably reduced. This coincides with the fact that those varieties that germinate poorly usually have a high percentage of their seed infected with bacteria. The bacteria involved are rod-shaped with peritrichous flagella, which should place them in the genus *Erwinia*.

Therapeutic Action of Vapors from Sulphur Compounds. C. E. YARWOOD. The vapors from dilute lime sulphur or commercial hydrogen sulphide gas diluted with air, have consistently killed out rust infections after symptoms of the disease were apparent, but without apparent host injury. Tests were conducted with bean rust, bean powdery mildew, clover powdery mildew, sunflower rust, cucumber powdery mildew, snapdragon rust, and snapdragon downy mildew. In preliminary tests detached mildewed clover leaflets sealed in chambers containing 3 cc. of test lime sulphur in 350 cc. of air space were used. In later tests potted infected plants under bell jars were subjected to a continuous flow of diluted hydrogen sulphide, or to air or carbon dioxide that had passed through 0.1 or 1 per cent lime sulphur. Hydrogen sulphide at 1 to 5000 at 10 l. per hr. destroyed 5-day-old pustules of bean rust and snapdragon rust without injury to the plants. Air passed through 0.1 per cent lime sulphur was nearly as effective as hydrogen sulphide. These sulphur-vapor treatments were effective against all rusts and powdery mildews tested but were relatively ineffective against snapdragon downy mildew.

Cultural Separation of some Obligate Parasites. C. E. YARWOOD. Downy mildews, powdery mildews, and rusts, may occur simultaneously on the same host, and it has not been possible to separate and grow them in pure cultures on artificial media, as is possible with most parasitic fungi. The inoculated or infected plants may, however, be subjected to treatments that will eliminate one or more of the parasites without greatly affecting the others, or without injuring the host. Beans inoculated simultaneously with powdery mildew and rust developed only powdery mildew when the plants were incubated in a dry environment, and developed only rust if sprayed with an eradicant fungicide, such as lime sulphur one day or more after inoculation and incubation in a moist chamber. The same treatments were effective in separating cucumber powdery mildew from cucumber downy mildew. Snapdragon plants simultaneously infected with downy mildew and rust were freed of active downy mildew by exposing them to the vapors of benzene or paradichlorobenzene, and were freed of rust by exposing them to the vapors of dilute lime sulphur. Benzene vapors also eradicated rust infections in some tests. Differential protective fungicides and differential toxic agents in the inoculum have also been used to separate these obligate parasites.

A Phycomycete Affecting Roots of Raspberry. S. M. ZELLER and A. J. BRAUN. A disease of Cuthbert raspberry prevalent in most raspberry-growing districts in Oregon causes the gradual death of plants. It attacks old and young plantings. The feeder rootlets are infected by a phycomycetous fungus, which may be an endotrophic mycorrhizal form during part of its life history. The fungus does not occur in plantings that show no disease. It has not been isolated in culture. Sterilized, diseased soil yielded 116 per cent more growth than nontreated, diseased soil. Such eradicants as ammonium sulphate and ammonium phosphate in excessive amounts yield much more growth in diseased soil than do the complete nutrients of recommended strength.

CHALAROPSIS THIELAVIOIDES, CAUSE OF "BLACK MOLD" OF ROSE GRAFTS

KARLA LONGRÉE

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INTRODUCTION

In 1938 and 1939, and probably earlier, rose growers and distributors in the Eastern States and the vicinity of Chicago were confronted with a rose trouble new to them. Severe damage was done to roses in the grafting frames by a disease that prevented formation of callus and "taking" of the grafts and caused the death of the scions involved. Always associated with this trouble was a fungus that covered the cut surfaces of the attacked grafts with its black growth. Losses were heavy and amounted, some growers claim, to nearly 100 per cent of their grafted plants.

In all cases reported thus far, the trouble could be traced back to shipments of American-grown stocks of the species *Rosa manetti* released from a small area in northern Oregon. It is important that, upon arrival, the plants usually had appeared, to the growers, healthy and vigorous. Such apparently healthy stocks, together with diseased grafts, were sent to the Department of Plant Pathology at Cornell University where they were used in this study. A preliminary report on the nature of the trouble and its cause has been presented recently under the name of "black mold of rose grafts" (7).

THE DISEASE

Newly infected rose grafts show a white to grayish-white, mildew-like fungous growth over the cut surface of both stock and scion, which is due to the mycelial growth of the pathogen and its profuse formation of endoconidiophores. The white color gradually turns to darker shades, partly because of the darkening of the endoconidiophores and endoconidia of the causal organism and also because of the formation of spores of a second type, the macroconidia, which range in color from hyaline (very young spores) to deep grayish-olive and fuscous (mature ones). The macroconidial type of spore gives the attacked rose graft its characteristic "moldy" aspect at the time it usually is removed from the frame. Scions affected by black mold show discoloration above the union (Fig. 1, A) which may later spread upward. Some discoloration was found in the stocks. The disease prevents the formation of a callus.

On nongrafted specimens of *Rosa manetti*, sent in from an eastern distributor, both types of conidia were found intermingled in abundance on all kinds of wounds or bruises with which the plants had become afflicted during the process of digging, packing, and shipment. Where the fungus had penetrated from the cut ends of branches, brownish discoloration of the affected stem ends, similar to that of invaded scions, was observed.

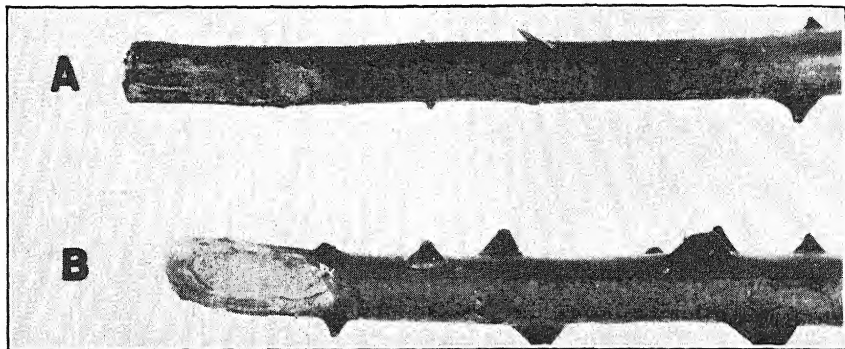


FIG. 1. A. Rose scion (variety Queen Mary) infected with *Chalaropsis thielavioides* in the grafting frame, following inoculation with endoconidia of the pathogen isolated from stem of *Rosa manetti*. B. Healthy, noninoculated control.

The disease seems mainly to be confined to grafted plants. Although examination of affected plants of *Rosa manetti*, shipped from Oregon, has, in a few instances, disclosed the pathogen on and in the tissue of injured roots, cases of a typical root rot have not been found. Leaves have never been reported as attacked, and, when artificially inoculated with the pathogen, have not become infected.

When invaded by the pathogen, the cells of cortex, wood, and pith of a rose stem vary somewhat in their type of reaction. The cortex, when attacked, shows a brown discoloration of both wall and content of its parenchyma and collenchyma cells. Discoloration is not so prominent in the wood and has not been observed in the xylem, although the mycelium and macroconidia of the pathogen were plentiful within the lumen of the vessels. The medullary rays, however, may become discolored following the invasion of the fungus. Discoloration also is pronounced in the small parenchyma cells of the pith. Observations in many instances have shown that the fungus may make considerable headway before any browning results. The fungus travels intracellularly. When infected stems are sectioned longitudinally, it appears that the fungus has made best advances in the vessels, while it grows only slowly through the cells of the cortex.

PATHOGENICITY

Inoculation tests were deemed necessary in order to find out whether the fungus constantly associated with the above-described grafting trouble was actually pathogenic on living rose tissue or merely a saprophyte. A clarification of this question seemed especially important in connection with the problem of effective control measures. Since the stocks were shipped east during the winter months, the possibility of a primary injury through frost—with the fungus following up as a saprophyte—could not be overlooked. Although sectioning of such plants had not revealed any symptoms of frost injury, additional evidence was desirable.

Scions of the varieties Queen Mary and White Killarney were grafted onto healthy stocks of *Rosa manetti*, *R. multiflora* and Gloire des Rosomanes ("Ragged Robin"); before grafting, the cut surfaces of both stocks and scions had been dipped in a suspension of endoconidia from a 3-day-old culture of the fungus previously isolated from infected specimens of *R. manetti*. Part of the grafts were left untreated and served as controls. The plants were then placed in moist sphagnum moss and kept at a temperature of 24° to 31° C. After 3 weeks, they were removed from the cases and inspected.

The control plants had callused well, and stocks and scions were united. The plants looked healthy, vigorous and free from fungous growth. All of the inoculated specimens showed symptoms typical of black mold. On the scions (Fig. 1, A) the discolored area was pronounced, varying from 2.1 cm. to 6.5 cm. in length. On the stocks, it varied from 0.1 cm. to 3.0 cm. in length. The cut surfaces of both scions and stocks were dark with macroconidia of the black-mold fungus and no callusing was noticeable. Sectioning of the specimens revealed the pathogen within the tissues of all inoculated plants.

In other tests, plants of *Rosa manetti* were simply wounded by cutting into their stems. Several drops of a suspension of young endoconidia were then placed on the fresh wound, which was kept moist with damp cotton and a dressing of Parafilm. After 2 days the wounded surface was white with young endoconidiophores that were producing great masses of endoconidia. After a week, young macroconidia were found intermingled with the endoconidia; after 3 weeks the cuts were black with macroconidia. There was no callusing, whereas the wounds of the controls showed considerable callus.

In still other experiments, healthy, vigorously growing plants of *Rosa*

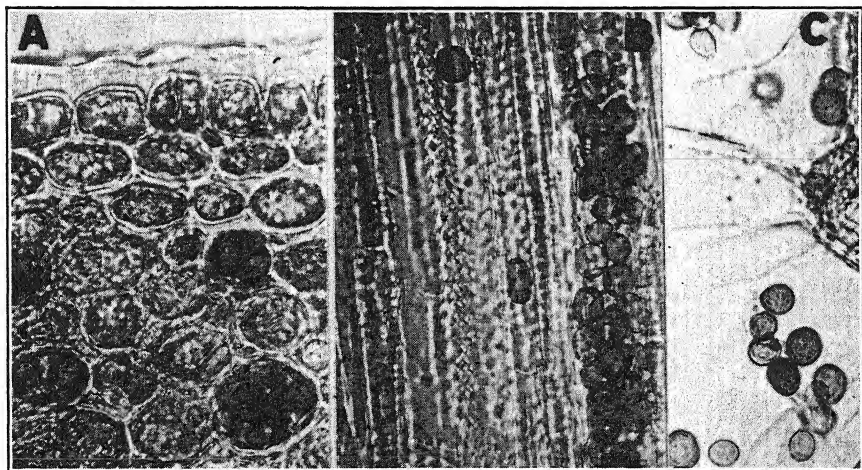


FIG. 2. A. Cross section through cortex of rose stem infected with *Chalaropsis thielavioides*. B. Longitudinal section through wood of same stem. C. Cross section through pith of same stem. All $\times 415$.

manetti were pruned and drops of a suspension of young endoconidia applied to the cut ends, which then were provided with a moist dressing, as described above. After a week the cut ends were definitely brownish, thus resembling naturally infected plants that had been shipped from the west. Sectioning of the discolored portion disclosed the mycelium and macroconidia of the pathogen in the cortex (Fig. 2, A), the wood (Fig. 2, B), and the pith (Fig. 2, C) of the rose stem. After 17 days the fungus had penetrated approximately an inch into a stem of 7 mm. diameter.

When healthy, uninjured rose stems were sprayed with a suspension of endoconidia and kept moist for 3 weeks, no infection resulted.

These experiments prove that the fungus always found associated with the grafting trouble is pathogenic on the rose. The infection court may be any wound, but it readily can be seen that the large wounds made in grafting serve as an ideal substrate and place of entrance for the fungus.

THE PATHOGEN

Taxonomy

The pathogen responsible for the black mold of rose grafts was identified as *Chalaropsis thielavioides* Peyronel, described and named in 1916 by Peyronel (9) in Italy, where it causes a stem rot of lupine. It has been held responsible for a similar trouble on lupine in Germany (8). Furthermore, this fungus has been found to cause a root rot and a graft disease of walnut in England (2, 3, 4) and a root rot of Chinese elm in the U. S. A. (5). It also has been isolated from peach seedling and carrot (3, 4).

At present there is only one species in the genus *Chalaropsis*, *Ch. thielavioides*; Hamond (4), however, has suggested that the isolate from carrot, which showed a considerable deviation from the type species as described by Peyronel, might represent a new species. Furthermore, Bliss (1) describes a root rot of date palms, caused by a *Ceratostomella* whose conidial stage was identified as a *Chalaropsis*, but not *Ch. thielavioides*, as understood by Peyronel. No technical description of this *Chalaropsis* on date has, however, been given by Bliss.

Chalaropsis thielavioides belongs to the family Dematiaceae; its perfect stage has not yet been found.

The similarity of the genera *Chalaropsis* and *Thielaviopsis* is striking. The writer feels that the relationship between *Ch. thielavioides* and *T. paradoxa* (de Seyn.) v. Höhn. (the species on which the genus *Thielaviopsis* was based) is even closer than that between *T. paradoxa* and *T. basicola* (Berk.) Ferraris. The conidia of *Ch. thielavioides* and *T. paradoxa* are morphologically very similar. The main difference between the two fungi lies in the manner in which their macroconidia are borne. In *T. paradoxa* the conidiophores are simple, short, lateral branches, perpendicular to the main hyphae. They may have 1-3 septa. The spores are formed at the tip of these conidiophores and hang together in chains. In *Ch. thielavioides* each macroconidium is borne singly and terminally; the spores are either sessile

or stand on short conidiophores. Usually, several spores occur together in a sympodial arrangement, as will be discussed later. *T. basicola*, however, differs considerably from *T. paradoxa*, as well as from *Ch. thielavioides*, in that its macroconidia (often referred to as "chlamydospores") are at first large, thick-walled, several-celled structures that, at maturity, break up into individual pill-box-like spores. Not included in this discussion is a third species of *Thielaviopsis*, *T. podocarpi* Petri, which has been described in Italy. The writer has not had access to the original publication and the notes published by Saccardo (10) and Lindau (6) were too meager to allow a comparison.

Morphology

The general morphologic characteristics of this rose pathogen answers well the description of the type species as given by Peyronel. The mycelium is septate, 2.2–5.4 μ wide, at first hyaline, later darkening to greenish shades. When growing under favorable conditions, on the host or in culture, the mycelium is somewhat sparse, and growth consists chiefly of endoconidiophores connected only by rather short hyphal threads. Another type of mycelium, also described by Hamond (4), is sometimes formed when the fungus meets adverse conditions. It is characterized by thin-walled, often vacuolated cells that may even swell up to form curious beadlike structures. This type of mycelium, however, must be regarded as abnormal.

The fungus produces 2 types of conidia—endoconidia² and macroconidia. The endoconidia are borne in endoconidiophores (Fig. 3, A). An endoconidiophore consists of 1–4 basal cells and the endoconidial cell proper in which the spores are formed and from which they are released. The endoconidiophores, as well as the mycelium, are at first hyaline, but soon their basal cells become greenish and most intensely so toward the base of the endoconidiophore. Measurements made on conidiophores developed on the host, *Rosa manetti*, indicated considerable variation in size. Basal cells varied in length from 9.3 μ to 17.6 μ and in width from 5.1 μ to 6.4 μ . The length of the endoconidial cell proper varied between 32 and 44.5 μ . Its width when measured toward the base varied from 5.1 to 6.4 μ , and, when measured at the tip, from 3.2 to 4.8 μ . Frequently, the lowest basal cell was found somewhat swollen and curved or hooked.

Endoconidia may be produced in great abundance, both on the host and in culture. They are formed within the upper portion—the "endoconidial cell"—of the conidiophore and expelled through its apex. There is but one spore at a time ready to be pushed out; but, often the remaining content of the endoconidial cell is already broken up into several segments, each of which will sooner or later become a spore. It seems that the wall of the conidiophore does not take any part in their formation. After the spores

² The author has decided to use this term because of its recognized standing (Snell, Walter H. Three thousand mycological terms. Publ. No. 2 of the Rhode Island Botanical Club, Providence, R. I. 1936). Actually, these spores are not true conidia because of their type of formation.

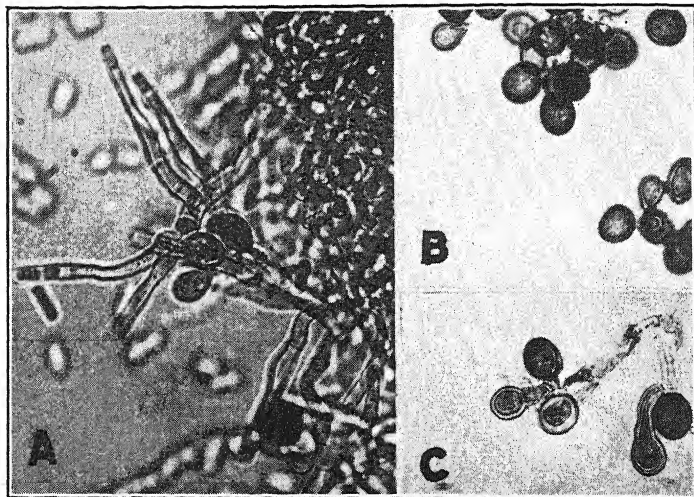


FIG. 3. A. Young endoconidiophores bearing endoconidia and young conidiophores bearing macroconidia of *Chalaropsis thielavioides* produced on rose stem at 24° C. under humid conditions. B. Macroconidia of same fungus. C. Young macroconidia of same fungus showing mode of their formation. All $\times 415$.

have left the endoconidial cell they often cling together in more or less long chains; 20 and more spores have been found united in such chains. Endoconidia are hyaline, biguttulate, and of cylindrical shape when "young," which here does not imply immaturity, since such spores may germinate well and normally. They vary considerably in size. With time endoconidia change their shape, becoming more or less oval. They also darken, taking on olive-greenish shades. Their average length decreases and their average width increases somewhat. It seems there is not so much variation in green spores as in hyaline conidia. Young, hyaline endoconidia, formed on the host, showed less variation than those formed on artificial substrates (Table 1).

The macroconidia are either sessile or borne on short conidiophores. Frequently these conidiophores arise from the base of the endoconidiophore. Under certain conditions the spores form terminally on the mycelium; this may take place on the above described abnormal mycelium. Macroconidia also may be formed terminally on hyphae below the surface of agar.

Macroconidia usually are borne in a sympodial arrangement. Often, the groups are so compact, due to the shortness of the conidiophores, that botryoid clusters result. Figure 3, C, indicates the acropetal succession in which these spores mature; those at the tips are the youngest. They are hyaline and here were stained with cotton blue. Macroconidia soon turn darker; and, although the wall of young macroconidia is thin, it thickens and eventually becomes a dark-brown perispore (Fig. 3, B). In mass, mature macroconidia appear olive-green to fuscous. While the young macroconidium contains numerous small droplets, the mature one is characterized by fewer and larger guttulae, which frequently coalesce into one enormous globule.

TABLE 1.—Comparative measurements, in microns, of conidia of *Chalaropsis thielavioides* occurring on and isolated from various host plants

Authority	Host	Endoconidia				Macroconidia			
		Length		Width		Length		Width	
		Extreme measurements	Average	Extreme measurements	Average	Extreme measurements	Average	Extreme measurements	Average
Peyronel, 1916 (Description of type)	Lupine	8.0-55.0	3.0-4.5	10.0-20.0	8.0-15.0
Hamond, 1935	Walnut graft (host)	7.5-16.5	12.4	3.5-5.5	4.5	12.5-21.0	16.8	10.0-14.5	12.6
"	Walnut graft (culture)	12.0-40.0	16.3	2.0-4.5	3.2	12.0-19.0	14.9	8.5-13.0	10.9
"	Walnut root	9.0-19.0	13.0	3.0-5.0	4.0	13.5-22.0	17.0	9.0-16.0	12.8
"	Walnut root (host)	11.0-30.0	17.1	2.0-4.5	3.1	14.0-19.5	17.4	9.5-14.0	12.1
"	Carrot (culture)	9.0-24.0	14.2	3.0-6.0	3.6	11.0-16.0	12.9	10.0-14.0	12.1
"	Peach seedling (culture)	12.0-32.0	19.6	3.0-5.5	4.4	13.0-23.0	17.0	9.5-14.5	12.0
Longrée	Rose stem (host)	8.0-16.8	11.2	3.2-4.8	4.0	9.6-16.8	13.4	9.6-14.0	11.5
"	Rose stem (yg. culture)	9.6-32.6	14.9	2.6-4.8	3.7	Only immature spores present			
"	Rose stem (old culture)	(Dark type of endoconidia) 7.0-19.2	11.9	4.2-7.4	5.6	9.6-17.0	13.5	8.0-15.4	11.3
"	Chinese elm root (host)	(No endoconidia present)				12.8-17.6	14.9	11.2-16.0	13.5
"	Chinese elm root (yg. culture)	(Hyaline type of endoconidia) 9.3-35.5	15.3	2.6-4.8	3.4	10.2-17.0	13.9	9.9-15.0	12.2
"	Chinese elm root (yg. culture)	(Dark type of endoconidia) 9.6-35.2	14.1	3.5-5.1	4.3

The macroconidia are subglobose to ovoid and possess a flattened base. They vary considerably in size. Measurements made in this study on conidia of *Chalaropsis thielavioides*, together with such made by other authors on the spores of this fungus, are combined in table 1. This does not include macroconidia still unseparated from the conidiophores, or spores having parts of conidiophores still attached to them.

Although there is considerable variation in the size of both kinds of conidia, of *Chalaropsis thielavioides* pathogenic on rose, the measurements agree rather well with those given by Peyronel for the type species. In fact, the variation as such seems even to be characteristic for that fungus.

The writer has not had an opportunity to compare Hamond's isolates with the fungus from rose, but has been able to study material of *Chalaropsis thielavioides* pathogenic on Chinese elm. The pathogen on elm differs somewhat, in size of conidia, from the form on rose, the macroconidial type of spores being a little larger in the elm fungus (Table 1). In culture, the endoconidia of the form from elm root tend to darken soon to an olive-green shade, while those of the rose fungus remain hyaline longer. When both fungi are allowed to grow under identical cultural conditions they readily can be distinguished. While the fungus from rose forms scant mycelium (Fig. 4, A), the isolate from elm makes a more profuse growth and produces more aerial mycelium (Fig. 4, B). Further differences, concerning rate of growth and pathogenicity will be discussed later.

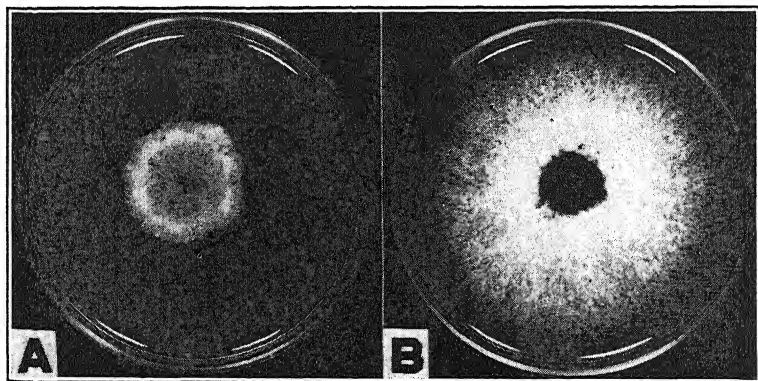


FIG. 4. A. Culture of *Chalaropsis thielavioides* isolated from stem of *Rosa manetti* growing on potato-dextrose agar, after three weeks at 18° C. B. Culture of *Chalaropsis thielavioides* isolated from root of Chinese elm growing on potato-dextrose agar, after three weeks at 18° C.

Physiology

Germination of Endoconidia.—Germination of endoconidia was studied under various temperature conditions. Hanging-drop cultures were prepared by placing drops of a suspension of spores in sterile distilled water on a sterile cover glass and inverting them over deep-well slides. Four different tests were run, the only variable factor being the age of the culture

from which the spores were used for the study. The results are presented in figure 5. Spores coming from a 2-day-old culture showed highest germinability. They not only reached a higher percentage of germination, but—a very interesting fact—their maximum temperature for germination was higher and their minimum temperature lower than for that of older spores. Furthermore, the range of their optimum germination was wider.

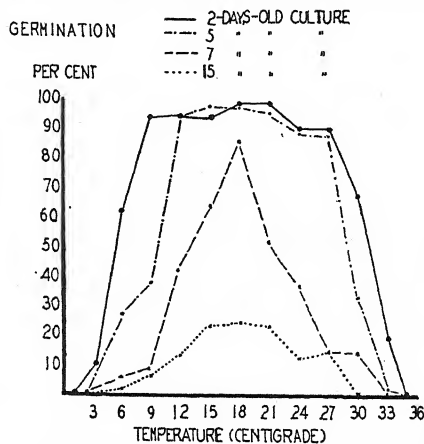


FIG. 5. Relation of temperature to percentage germination of endoconidia of *Chalaropsis thielavioides* from rose stem.

The time necessary for germination varied with the age of the spores and the temperature to which they were exposed. Conidia coming from a young (2-day-old culture) started germinating soon after they had been suspended in water (Table 2). After 24 hours, those exposed to temperature from 12° to 33° had reached their approximate maximum percentage of germination. After 2 days no further appreciable increase in germination was encountered at any temperature. Spores from old cultures germinated slowly, especially at the lower temperature range; there, highest counts were not found until after several days of exposure.

Under the conditions of these experiments endoconidia often germinated directly into endoconidiophores, provided that the spore material was taken from actively growing cultures and that the temperature was within or close to the optimum range of germination. Such newly-formed endoconidiophores were able to give rise to a new crop of endoconidia (Fig. 6, B). Below and above the range of temperature for optimum germination, endoconidia tended to form germ tubes (Fig. 6, A and C). At high temperatures, beginning with a temperature of 27° C. the spores frequently became swollen to such a degree as to appear spherical (Fig. 6, C); at 30°–33°, germ tubes, if formed, were often vestigial and abnormal.

Germination of Macroconidia.—Germination of macroconidia was studied with the same technique as that used in germinating the endoconidia. It became evident, however, that young cultures of the fungus could not be used in these tests because macroconidia evidently mature very slowly or,

TABLE 2.—*Relation of temperature to the germination of endoconidia of Chalaropsis thielavioides. (Spores from 2-days-old culture. 200 spores counted)*

Hours of exposure	Percentage germination at given temperature (° C.)										
	3-4	6-6.5	9-10	11-12	15-15.5	18-19	21-21.5	24-26	26-27	30-31	32-33
2	0.0	2.5	3.6	4.1	14.0	57.0	41.0	36.0	24.1	16.2	3.3
4	3.0	4.5	8.0	30.0	42.5	85.0	77.5	62.5	53.0	37.0	14.1
24	9.3	26.3	75.5	91.5	95.0	96.8	96.5	89.6	90.3	67.5	20.0
48	9.5	57.0	91.8	94.0	94.9	98.3	98.1	90.3	91.0	68.0	19.8
72	10.1	57.1	94.0	94.2	94.9	98.0	98.2	90.0	90.3	67.5	19.7

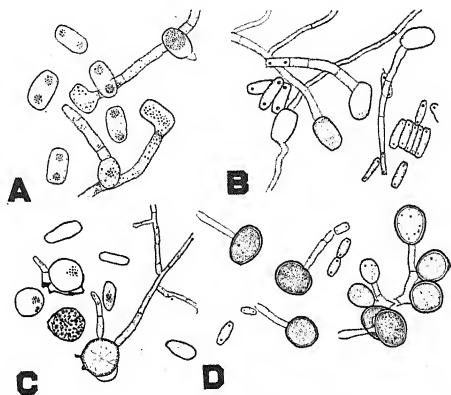


FIG. 6. A-C. Camera-lucida drawings of endoconidia of *Chalaropsis thielavioides* from stem of *Rosa manetti* germinating in distilled water over deep-well slides. A. Germination at 3°-5° C. B. Germination at 18° C. C. Germination at 32°-33° C. D. Camera-lucida drawings of macroconidia of same fungus germinating in distilled water over deep-well slides at 15° C. All $\times 320$.

when matured, seem to need a period of rest before they are able to germinate. Macroconidia, originating from a 2-month-old culture that had been kept at room temperature, did not germinate, while the spores derived from 3-month, 5-month and 7-month-old cultures germinated to some extent, although never so well as did the endoconidia. The results of some of these tests are presented in figure 7. The optimum temperature for the germina-

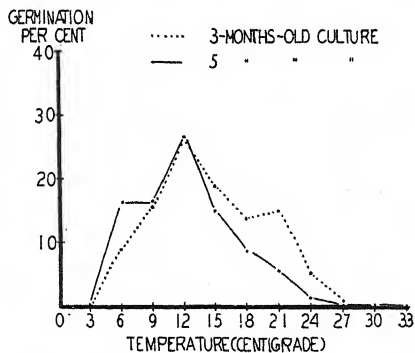


FIG. 7. Relation of temperature to percentage germination of macroconidia of *Chalaropsis thielavioides* from rose stem.

tion of macroconidia was 12° C., which is somewhat lower than that for endoconidia. The same is true for the maximum temperature for germination, which is between 27° and 30° C., depending on the germinability of the spore material. The minimum temperature for the germination of macroconidia was approximately the same as that for germination of "old" endoconidia, namely between 3° and 6° C. In many instances macroconidia germinated directly into endoconidiophores (Fig. 6, D), which, in turn, produced endoconidia. This was found to take place at temperatures from 6° to 27° C. As a rule, the germ tube or endoconidiophore—as the case

may be—was put forth laterally; but, in some cases, germination took place from the flattened “base” of the macroconidium.

The process of germination was much slower in the macroconidium than in the endoconidium. Hamond (4), working with macroconidia of *Chalaropsis thielavioides*, which originated from “freely-growing cultures,” states that a resting period of 4 days was required, after which germ tubes were produced by such spores taken from the peach, carrot, and walnut-root cultures; a period of even 5 days had to elapse before spores from the walnut-graft culture had begun to germinate. In the present study on the isolate from rose it was observed that macroconidia had germinated to some extent within the first 24 hours of exposure, at a temperature range from 12° to 24° C. But, even at temperatures most favorable for germination, a period of 7–10 days was necessary for maximum germination.

Growth.—A number of tests were made in order to study the effect of temperature on the growth of *Chalaropsis thielavioides*, pathogenic on rose. In one of these, the isolate from elm root was compared with the one from rose.

In each case, 1.5 per cent potato-dextrose agar (pH 5.6 to 5.8) was used. The plates were seeded, sealed with Parafilm, and incubated for 24 hours at 24° C. They were then distributed into the different temperature chambers, the temperatures ranging from 0° to 35° C. After certain intervals of time, measurements were made of 2 diameters at right angles to each other, and, after 35 days, each test was terminated. The results of the experiment on the effect of temperature on growth of the isolates from rose are presented in figure 8. The range for optimum growth was found to be

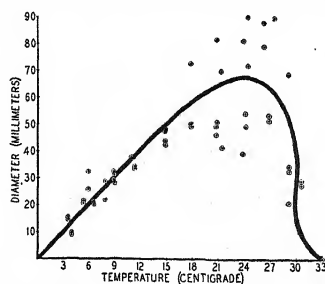


FIG. 8. Average curve of growth in 35 days of *Chalaropsis thielavioides* isolated from stem of *Rosa manetti*. On potato-dextrose agar.

between 18° and 27.5° C.; the minimum temperature was found to lie between 0° and 3.5° C. and the maximum, from 30.5° to 33° C. Not only had no growth taken place on the plates incubated at 33° C., but the inoculum had even lost its viability after having been exposed to that temperature for the above-mentioned period (35 days). At all temperatures where growth took place, mycelium was sparse, while endoconidiophores bearing endoconidia were produced in abundance. All cultures appeared white at first, but sooner or later—depending on the temperature—macroconidia were formed, beginning in the center of the plate. The white color thus changed gradually to greenish shades and eventually to a very dark fuscous. The

formation of endoconidia was restricted to the surface of the substrate, while the macroconidia were formed on, as well as within, the agar. At lower temperatures, the outline of the thallus was smooth, whereas at higher temperatures—beginning at 18° C.—it was more or less lobed, especially at the temperature range from 21°–27.5° C. Although the inoculum for the tests was taken from a culture originating from a single endoconidium, variation in the extent of growth was considerable at higher temperatures (Fig. 8). At 27° C. and above the presence of abnormal, thin-wall mycelium and of swellings, as described above, was observed.

COMPARISON OF GROWTH OF ROSE FUNGUS AND ELM FUNGUS

Since the pathogen from elm had an appearance in culture decidedly different from the fungus isolated from rose (Fig. 4), a comparative test of the 2 strains was run according to the above-described technique. The inoculum came from cultures of identical age. Results of this experiment are presented in figure 9. It is surprising how similar the cardinal points:

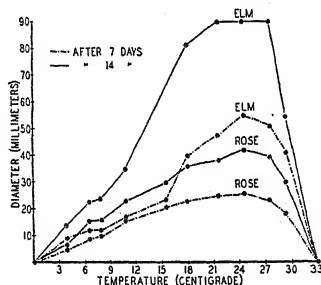


FIG. 9. Average curves of growth in one and two weeks of *Chalariopsis thielavioides* isolated from stem of *Rosa manetti* and root of Chinese elm. On potato-dextrose agar.

optimum, maximum, and minimum, are for the two strains. But the elm strain has a much faster growth rate than does the isolate from rose. The same holds true in regard to the formation of macroconidia, which started earlier and took place more rapidly in the elm fungus.

Growth of the Fungus on Substrates Other than Potato-dextrose Agar

The isolate from rose was able to grow and fruit for a while (in moist chamber) on nonsterilized pieces of wood from Chinese elm, black walnut, English walnut, poplar, and peach, as well as on raw potato tuber and carrot. In no instance did the fungus penetrate deeply into any of these tissues, and growth remained entirely superficial.

The isolate from Chinese elm grew and fruited for a short while on the surface of nonsterilized pieces of rose stem (moist chamber). It also developed to some extent on the cut stem ends of rose plants growing in the greenhouse, but failed to penetrate deeply into living rose stems; finally, the wounds callused over and healed.

Odor

Chalaropsis thielavioides, isolated from rose, when growing freely in culture or when fruiting on rose tissue in moist chambers, was found to give off a characteristic, sweet, fruity odor, resembling that of isobutyl acetate.³ A similar odor, although much weaker, was produced by the isolate from elm root when growing in culture. Cultures of various isolates of *Thielaviopsis paradoxa* (de Seyn.) v. Höhn. (= *T. ethacetica* Went), which is well-known for its pineapple-like odor (11), were compared with both isolates of *Ch. thielavioides*. Although the odor from cultures seemed very much alike in type, there were definite differences in strength. By far the most powerful odor was given off by the fungus from rose.

LIFE CYCLE AND CONTROL

With the facts in hand we can, at present, say little or nothing about the life cycle of the pathogen. We do not know the perfect stage of the fungus, nor possible additional hosts; and it seems that a study of the life cycle of the fungus remains a problem to be worked out at the place where the infected and infested plants originated. This is especially desirable, since the black mold of rose grafts is not yet to be regarded as well established in eastern greenhouses and, since, up to the present, every outbreak of the trouble has followed the use of stocks grown in a small, restricted area in Northern Oregon. Since correspondence with F. P. McWhorter, Oregon State College of Agriculture, shows, on the other hand, that in the nurseries no indication of infection of the plants in the fields could be detected, one can now—with only few facts in hand—merely theorize on the possibilities of the infestation or infection of those Oregon-grown *Rosa manetti* plants. If they were not infected in the field, they still might have been infested with contaminated soil adhering to them so that, under conditions favorable for the development of the fungus, infection took place later, perhaps in storage, during transportation, or in the grafting frame. Aside from the soil in the field, the storage house might be suspected as having been contaminated; or the fungus may have been carried with packing material in which the plants were shipped. At any rate, examination of the specimens of *Rosa manetti* under discussion has shown that they were infested and also partly infected when they reached the eastern distributor and grower.

The problem of control obviously rests with the nurserymen who grow roses to be used as stocks in grafting. Once it has become an established fact that the pathogen is carried in and with the plants used for grafting, any rose grower, buying stocks, will in the future insist on clean and healthy material.

No tests dealing with control have been made in the present study, but work done by Hamond (4) and practical experience common to growers in

³ Thanks and credit are due to Mr. E. C. Crocker, of the Arthur D. Little Company, Cambridge, Mass., for his valuable help in identifying the type of odor produced by the fungus, and to Dr. D. E. Bliss, Citrus Experiment Station, Riverside, Calif., for making the cultures of *Thielaviopsis paradoxa* available to the author.

this country indicate that the problem should not be a difficult one. Disinfestation of the stocks before shipping seems to be a simple and promising control measure, since *Chalaropsis thielavioides* is easily killed by various disinfectants.

SUMMARY

A disease of rose grafts that has recently alarmed rose growers is described. This disease prevents the formation of callus and the "taking" of grafts and causes the death of the scions involved. The cut surfaces of the grafts are covered with the mycelium and spores of the pathogen. This may also penetrate deeply into the tissue of both stocks and scions.

The causal organism is *Chalaropsis thielavioides* Peyronel; its pathogenicity on rose has been demonstrated.

The morphology of the pathogen is described and the results of studies on the effect of temperature on the fungus are presented.

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RELATION OF CROWN-RUST INFECTION TO YIELD, TEST WEIGHT, AND LODGING OF OATS¹

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INTRODUCTION

The reduction in oat yields in Iowa from crown rust (*Puccinia coronata avenae* Eriks.), in the 20 years from 1919 to 1938, was more than twice that caused by stem rust (*P. graminis avenae* Eriks. and Henn.). Prior to the distribution of stem-rust-resistant varieties, such as Richland (Iowa 105), in 1914, and Iogold, in 1926, stem rust possibly caused more damage to the Iowa oat crop than did crown rust. Unusually severe epiphytotics of crown rust occurred in Iowa in 1935 and 1938, which caused estimated yield reductions of 20 and 24 per cent, respectively.

In 1938, 283 varieties and selections of oats were grown in replicated yield tests at Ames, and 159 in similar tests at Kanawha. These varieties and selections, mostly of hybrid origin, ranged in resistance to crown rust from the near immunity of the parent variety Bond to the high susceptibility of such parental varieties as Markton and Iogold. Data obtained on these selections at Ames and Kanawha included percentage and type of crown-rust infection, per cent lodged, date of ripening, height, grain yield, and test weight. The interrelationships of these characters were studied with particular reference to crown-rust infection, yield, and test weight.

REVIEW OF LITERATURE

Immer and Stevenson (6), Immer and Ausemus (5), and Greaney (3, 4) have reported statistical studies on the effect of crown rust on yield, test weight, and other characters of oats grown in the field. Immer and his co-workers, who used data obtained from yield tests involving 29 to 280 strains of oats grown at 1 to 4 Minnesota stations from 1927 to 1929, found consistent negative correlations of $-.18$ to $-.51$ between percentage of crown rust and yield. They found that plumpness of grain, date of heading, crown-rust resistance, and lodging were closely associated with yield, while height of plant showed very little association with yield. Immer and

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Stevenson (6) found that the partial regression of yield on percentage of crown-rust infection, with height, heading date, and lodging held constant, was $-.32$ for 280 oat strains grown at St. Paul, Minnesota, in 1927.

Greaney (4) used sulphur dust to control crown rust near Winnipeg, Manitoba, in 1930, and in 86 plots of Victory having a range in crown-rust infection from 2 to 76 per cent and also severely infected with stem rust, the correlation between crown rust and yield was $-.90$. He did not determine the regression of yield on crown rust, but for each 1 per cent increase in stem rust the yield was decreased by an average of 0.47 bushel per acre. In 1931, a 45 per cent infection of crown rust reduced the yield of the stem-rust resistant strain of Hajira \times Banner 4.8 bushels per acre.

Murphy (9, 10, 11) reported previous studies on the effect of crown rust on yield, water requirement, chemical composition, and cold resistance of oats.

MATERIAL AND METHODS

The 442 varieties and selections used in this study were distributed among the 1938 Iowa yield tests as follows: 80 at Ames in 2 replications of single 20th-acre plots, 78 at Ames in 4 replications of single rod rows, and 125 at Ames and 159 at Kanawha with 10 rod rows of each distributed into 1 replication of 4-row plots and 3 replications of 2-row plots. All replications were arranged systematically. Yield was recorded for each plot or row, while test weight, ripening, height, lodging, and crown rust were recorded as an average for all replications. Yield was expressed in bushels per acre, test weight in pounds per bushel, date ripe in days (in July), height in inches, and lodging in percentage of culms lodged with some weight given to degree of lodging.

The degree of crown-rust infection was determined by a comparison with the scale for estimating rust percentages adopted by the Division of Cereal Crops and Diseases (2). The degree of susceptibility of each variety and selection was recorded by assigning numerical equivalents of 0.1 to 1.0 to the types of infection observed under field conditions, as previously described (8). Coefficient of crown-rust infection was then calculated by multiplying the numerical equivalent by the percentage of infection. Levine, Stakman, and Stanton (7) used a similar method in expressing a coefficient of stem-rust infection on oats, but thought that the degree of susceptibility to crown rust was expressed accurately by the percentage figure. The percentage of infection on the varieties and selections observed in this study ranged from 0 to 100, and every type of infection (numerical equivalents 0.1 to 1.0) was observed.

Approximately half of the varieties and selections grown in 20th-acre plots were of hybrid origin. Nearly all of the varieties and selections grown in nursery tests were selections from crosses between high-yielding, stem-rust-resistant varieties, such as Richland, Iogold, and Rainbow, and the crown-rust-resistant and smut-resistant varieties, Victoria and Bond. Mark-

ton, also, was used as a source of resistance to smut. The breeding methods used with some of the crosses were described previously (1, 12, 15). Selections definitely known to be inferior in yielding ability, in resistance to either rust or either smut, or in stiffness of straw, had been discarded. The selections used in this study, therefore, had undergone considerable selection before 1938, and many selections known to be highly susceptible to crown rust had been previously discarded. Many more selections were discontinued after 1938, but the data from all of the selections grown in replicated tests in 1938 are included in the study reported here.

Statistical methods described by Snedecor (13) were used throughout this study. The total correlation coefficient expresses the degree in which two variables tend to be associated in value. The partial correlation coefficient is used to express the degree of relationship between two characters, with the other observed characters held constant. The multiple correlation coefficient measures the combined relation between a dependent variable and a series of independent variables. The partial regression coefficient is used to express the effect of one character on another, with other known variables held constant, *e.g.*, average decrease in yield for one unit increase in coefficient of crown-rust infection. Standard partial regression coefficients express the relative independent effects of different variables on a dependent variable in standard units of measurement. The simple regression coefficient expresses the increase or decrease in the dependent variable for one unit of increase in the independent variable without consideration of other observed characters.

RESULTS OBTAINED

A Comparison of Methods of Estimating Degree of Crown-rust Infection

Percentage of infection indicates the relative area of the leaves and leaf sheaths affected by crown rust. It does not take into consideration differences in type of infection. In a breeding nursery, type of infection would appear to be fully as important as percentage of infection. As previously described, both values are arbitrarily combined in the calculated coefficient of infection. A study was made to determine which of the three values showed the highest correlation with yield. The total correlations of percentage, type, and coefficient of infection with yield, test weight, date ripe, height, and lodging of the 80 varieties and selections included in the 20th-acre plots at Ames, and, with yield of the 125 and 159 varieties and selections at Ames and Kanawha, respectively, are presented in table 1.

Regardless of the method used for evaluating crown-rust infection, all of the correlation coefficients between crown rust and yield and between crown rust and test weight were highly negative and highly significant. None of the correlations in table 1 between crown rust and ripening date, height, and lodging is significant. Coefficient of infection was more highly correlated with yield and test weight than were either of its components

(percentage or type of infection), and percentage of infection was more closely associated with yield and test weight than was type of infection. On the basis of the computations presented in table 1, coefficient of infection is most closely related to yield and was used in subsequent calculations. It is possible that some weighted values for type and percentage of infection by crown rust might give a closer relation to yield than a simple product of the two estimates.

TABLE 1.—*Correlation between crown-rust infection (expressed in percentage, type, and coefficient) and yield, test weight, lodging, height, and date ripe, of oat varieties and selections grown at Ames and Kanawha, Iowa, in 1938*

Location and characters correlated	Varieties and selections	Total correlation coefficients ^a with crown rust expressed in		
		Percentage (0-100)	Type (.1-1.0)	Coefficient (0-100)
Ames:	Number 80			
Crown rust with				
Yield		-.73	-.75	-.80
Test weight		-.68	-.64	-.74
Date ripe		-.03	-.05	-.03
Height		+.11	+.22	+.16
Lodging		+.12	+.07	+.21
Ames:	125			
Crown rust with				
Yield		-.74	-.64	-.79
Kanawha:	159			
Crown rust with				
Yield		-.77	-.74	-.77

^a Highly significant coefficients (beyond 1% point) are printed in boldface type.

Relation of Crown-rust Infection to Yield and Other Agronomic Characters

Average Yields of Crown-rust Infection Groups. The average yields of the varieties and selections grown in the 4 tests at Ames and Kanawha, grouped according to coefficient of crown-rust infection, are shown in table 2. With few exceptions, the yields decrease with an increase in crown-rust infection. The varieties and selections do not represent a normal distribution for rust infection because of previous selection for yield and rust resistance and the inclusion of standard varieties known to be susceptible. Nevertheless, the striking negative relationship between yield and crown rust is obvious.

The low average yield of the varieties and selections in 20th-acre plots was due in part to the inclusion of about 20 standard varieties that were susceptible to crown rust. One selection in the 20th-acre plots (Morota × Bond, C. I.³ 3514) was completely resistant to crown rust, but, unfortunately, its straw was very weak. The 78 selections grown in replicated single-row plots were sown late, and this may account in part for their lower average yield in comparison with the other selections grown at Ames.

³ C. I. refers to accession number of the Division of Cereal Crops and Diseases.

TABLE 2.—Yields of oats at Ames and Kanawha, Iowa, in 1938, grouped according to coefficients of crown-rust infection

Location	Varieties and selections	Size of plots	Average yield in bushels per acre with crown-rust coefficient of ^a										Average		
			0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Acre yield	Coef. crown rust
mes	<i>Number</i>	20th acre	(1) 64.7	(5) 67.4	(6) 63.9	(6) 52.8	(7) 47.4	(13) 39.2	(13) 37.4	(22) 36.0	(6) 38.1	(1) 31.3	<i>Bushels</i> (80) 43.5	49.1
mes	80	Rod rows	(3) 75.5	(39) 69.8	(3) 63.5	(5) 58.8	(8) 55.7	(5) 48.1	(10) 36.7	(1) 37.1	(2) 30.2	(2) 24.9	(78) 59.4	25.2
mes	125	Rod rows	(12) 79.9	(53) 71.7	(16) 68.2	(6) 65.2	(1) 58.4	(6) 48.8	(5) 53.2	(5) 46.7	(12) 44.2	(9) 40.0	(125) 63.8	26.7
anawha ..	159	Rod rows	(12) 81.9	(53) 70.3	(18) 67.3	(10) 61.0	(11) 61.5	(4) 51.8	(12) 42.2	(19) 43.5	(20) 38.7	(159) 59.9	30.1
total	442	(28) 79.7	(150) 70.6	(43) 66.9	(27) 59.7	(27) 56.0	(23) 43.9	(35) 42.8	(56) 39.6	(39) 40.3	(12) 37.6	(2) 24.9	(442) 58.0	31.7

^a Figures in parentheses indicate number of selections or varieties in the group.

Relation of Yield to Crown-rust Infection in Different Hybrid Groups.

The varieties and selections in all 4 tests are classified in table 3 according to hybrid combination or origin and then grouped into yield classes. The selections from crosses involving the Bond variety had the least crown rust and the highest yields. Twenty-eight of the 38 strains were highly resistant to crown rust and yielded an average of 79.7 bushels. The indicated high yield of the one selection in the 31-to-40 crown-rust class may be an error. The performance of the Bond hybrids demonstrates the value of that variety as a source of resistance to crown rust. This group of selections also was resistant to most races of stem rust and both smuts, as reported by Murphy *et al.* (12).

The 143 selections from the Victoria \times Richland cross had an average yield of 71.9 bushels and an average crown-rust coefficient of 6.7. Selections from this cross were highly promising from the standpoint of stem rust, loose smut, and covered smut, as well as yield and resistance to crown rust. They had been selected closely for crown-rust resistance (15). Among these selections 122 were in the 1-to-10 crown-rust class, and none had a coefficient above 40. One selection from this cross, C. I. 3305, was recently distributed by the Iowa Agricultural Experiment Station under the name of Boone. Boone had an average crown-rust coefficient of 9 and an average yield of 68.7 bushels at Ames and Kanawha in 1938.

The history of the Markton \times Rainbow cross was reported previously (1). Certain of the selections from this cross were very outstanding for yield, high test weight, and resistance to stem rust, both smuts, and certain races of crown rust. Two selections, C. I. 3247 and 3346, were named Marion and Hancock, respectively, and were recently distributed from the Iowa station. The description, history, and registration of these two varieties have been reported previously (14). The average crown-rust coefficients for Marion and Hancock in 1938 were 21.3 and 59.8, with average yields of 58.8 and 56.0 bushels, respectively. These two, as well as other selections from this cross, have produced higher yields and heavier test weights than would be indicated by the crown-rust coefficients.

Among the 45 selections from miscellaneous crosses, 29 with coefficients between 1 and 30 were from crosses involving Victoria. Some of the remaining were from crosses in which none of the parents was resistant to crown rust.

Since both Iogold and Markton are susceptible to crown rust, all selections from the Markton \times Iogold cross were more or less susceptible. Certain of the Markton \times Iogold selections were outstanding for yields, in the absence of crown rust. They also were highly resistant to stem rust and to both smuts. The standard varieties grown were mostly those used as parents in the different crosses or as check varieties. The average coefficients of crown rust recorded for the stem-rust-resistant parent varieties Rainbow, Morota, Richland, and Iogold were 22, 27, 68, and 75, respectively. The smut-resistant varieties Bond, Victoria, and Markton were not included in the 1938

TABLE 3.—Yields and crown rust infection of groups of oat selections and varieties at Ames and Kanawha in 1933

Source of selections or varieties	Average yield in bushels per acre with crown-rust coefficient of ^a										Average		
	0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Acre yield	Coef. crown rust
Bond hybrids	(28) 79.7	(1) 73.2	(3) 54.6	(4) 46.3	(2) 52.0	(38) 72.6	1.6
Victoria × Richland	(122) 72.3	(18) 71.6	(2) 61.1	(1) 54.1	(143) 71.9	6.7
Markton × Rainbow	(2) 66.9	(20) 66.6	(21) 60.7	(23) 56.4	(12) 44.0	(7) 42.1	(1) 65.4	(1) 31.3	(87) 57.0	31.8
Misc. crosses	(26) ^b 62.8	(2) ^b 57.4	(1) ^b 53.5	(3) 63.6	(3) 43.3	(4) 38.0	(4) 34.2	(2) 24.9	(45) 54.7	30.7
Markton × Iogold	(2) 32.7	(9) 43.0	(17) 40.0	(10) 39.4	(1) 44.8	(39) 40.3	65.4
Standard varieties	(3) 52.6	(3) 51.1	(2) 43.8	(6) 42.1	(16) 36.7	(31) 37.4	(23) 40.0	(6) 39.8	(90) 39.5	63.9
Total	(28) 79.7	(150) 70.6	(43) 66.9	(27) 59.7	(27) 56.0	(23) 43.9	(35) 42.8	(56) 39.6	(39) 40.3	(12) 37.6	(2) 24.9	(442) 58.0	31.7

^a Figures in parentheses indicate number of selections or varieties in the group.^b Selections from single or multiple crosses in which Victoria was used as one parent.

replicated yield tests, but in similar single rod-row tests their crown-rust coefficients were 0, 6, and 95, respectively.

Correlation between Crown-rust Infection, Yield, and Other Agronomic Characters.—Despite the fact that considerable selection for certain of the characters studied was made during earlier segregating generations, it seemed worth while to determine the total correlations between the 6 characters studied, viz., reaction to crown rust, yield, test weight, date ripe, height of plants, and amount of lodging. The total correlation coefficients representing all combinations between the 6 variables are presented in table 4.

Yield and test weight were both strongly negatively correlated with coefficient of crown rust infection in all tests. The total negative correlations between crown rust and yield were consistently higher, however, than those between crown rust and test weight. This is true also of the partial correlations $r_{YC \cdot WDHL}$, which measures the relationship between yield and crown rust when the effects of test weight, date ripe, height, and lodging are held constant; and of $r_{WC \cdot YDHL}$, which measures the relationship between test weight and crown rust when the effects of yield, date ripe, height, and lodging are held constant. The values of $r_{YC \cdot WDHL}$ for the groups of 80, 78, 125, 159, and 442 varieties and selections were $-.59$, $-.45$, $-.69$, $-.49$, and $-.67$, respectively, while the corresponding values of $r_{WC \cdot YDHL}$ were $-.33$, $-.10$, $-.01$, $-.28$, and $-.15$.

TABLE 4.—Total correlation between crown rust, yield, test weight, lodging, height, and date ripe, of oat varieties and selections grown at Ames and Kanawha in 1938

Characters correlated	Total correlation coefficients ^a				
	Ames			Kanawha	Total
	(80) ^b	(78)	(125)	(159)	(442)
Crown rust with:					
Yield	-.80	-.75	-.79	-.77	-.77
Test weight	-.74	-.67	-.58	-.71	-.68
Date ripe	-.03	-.27	-.36	-.42	-.27
Height	+.16	+.27	-.03	+.11	+.10
Lodging	+.21	+.14	+.11	+.37	+.23
Yield with:					
Lodging	-.32	-.39	-.17	-.52	-.38
Height	-.38	-.24	-.08	-.05	-.13
Date ripe	-.23	+.09	+.10	+.16	+.05
Test weight	+.74	+.86	+.70	+.87	+.80
Test weight with:					
Lodging	-.13	-.33	-.13	-.55	-.34
Height	-.13	-.02	+.18	+.08	+.01
Date ripe	-.08	+.30	+.21	+.14	+.09
Lodging with:					
Date ripe	+.36	+.31	+.32	-.01	+.15
Height	+.49	+.52	+.37	+.23	+.36
Date ripe with:					
Height	+.54	+.53	+.62	+.29	+.44

^a Highly significant coefficients (beyond 1% point) are printed in boldface type.

^b Numbers in parentheses refer to number of varieties and selections.

Except for yield and test weight, the total correlation coefficients between crown rust and the other observed characters were not consistently highly significant. Crown rust had a highly significant, negative correlation with date ripe among the 125 and 159 selections grown at Ames and Kanawha, respectively, and among the entire 442 varieties and selections. The total correlation coefficients between lodging and crown rust were all positive, but highly significant only at Kanawha and among the entire 442 varieties and selections. Crown rust did not show a highly significant association with height in any instance, but the relationship tended to be positive.

Yield was negatively associated with lodging and height, and positively associated with date ripe and test weight. The highly significant positive correlations between yield and test weight were about equal in magnitude to the negative correlations between yield and crown rust.

The multiple correlation coefficients of yield with crown rust, test weight, lodging, height, and date ripe for the groups of 80, 78, 125, 159, and 442 varieties and selections were $R = .88, .92, .87, .90$, and $.90$, respectively. For the 442 varieties and selections it may be said that 81 per cent ($R^2 = .81$) of the total squared variability in yield was attributable to the 5 characters studied, leaving 19 per cent unexplained or due to random variation. The multiple correlation coefficients of test weight with crown rust, yield, lodging, height, and date ripe for the groups of 80, 78, 125, 159, and 442 were $R = .79, .91, .75, .89$, and $.82$, respectively.

Lodging tended to be associated with the later and taller types of oats at Ames, but only with tallness at Kanawha. As might be expected, the taller oats tended to mature later, the correlation between date ripe and height ranging from $+ .29$ to $+ .54$.

The standard partial regression coefficients (betas) of yield and test weight on each other, and on coefficient of crown rust, date ripe, lodging, height, and bushel weight are presented in table 5. These coefficients measure the average effect of each of the observed characters on yield and test weight in units of standard measure. The betas are directly comparable and their magnitude indicates the relative effect of the observed characters on yield and test weight, with the other observed characters held constant.

The partial regression coefficients indicate the average effect of each observed character on a dependent variable in terms of the units of measure used in recording the data. The partial regression equations for estimating yield in the groups of 80, 78, 125, 159, and 442 varieties and selections were as follows (Y, W, L, H, D = yield, test weight, lodging, height, and date ripe, respectively) :

$$\begin{aligned}
 (80) \quad \bar{Y} &= -.31C - .02L - .26H - .23D + .93W + 60.14 \\
 (78) \quad \bar{Y} &= -.21C - .04L - .10H - 2.00D + .47W + 82.42 \\
 (125) \quad \bar{Y} &= -.32C + .03L - .29H - 1.11D + 1.91W + 52.71 \\
 (159) \quad \bar{Y} &= -.27C - .08L + .62H - .45D + 2.39W + 59.84 \\
 (442) \quad \bar{Y} &= -.29C - .05L - .02H - .44D + 2.24W + 26.29
 \end{aligned}$$

In 1938, yield apparently was determined primarily by the amount of crown-rust infection and test weight—either one alone having more effect on yield than the combined effects of date ripe, lodging, and height. For each unit increase in coefficient of crown rust, in the presence of the other observed characters, yield was decreased an average of 0.21 to 0.32 bushels per acre. For each pound increase in test weight yield was increased an average of 0.47 to 2.39 bushels per acre. For each day's delay in maturity yield was decreased an average of 0.23 to 2.00 bushels per acre. The effect of lodging and height on yield was neither consistent nor generally highly significant.

TABLE 5.—Standard partial regression (betas) of yield and test weight on crown rust, lodging, height, and date ripe in oat varieties and selections grown at Ames and Kanawha in 1938

Characters	Standard partial regression coefficients ^a				
	Ames			Kanawha	Total
	(80) ^b	(78)	(125)	(159)	(442)
Standard partial regression of yield on:					
Crown rust coefficient	-.54	-.33	-.64	-.44	-.50
Date ripe	-.12	-.18	-.18	-.13	-.11
Lodging	-.04	-.05	+.04	-.11	-.08
Height	-.17	-.02	-.07	+.12	-.01
Test weight	+.30	+.68	+.39	+.50	+.50
Standard partial regression of test weight on:					
Crown rust coefficient	-.39	-.08	+.01	-.27	-.14
Lodging	+.07	-.17	-.11	-.20	-.08
Height	+.13	+.19	+.27	+.16	+.12
Date ripe	-.08	+.16	+.01	-.12	+.04
Yield	+.49	+.77	+.71	+.57	+.90

^a Highly significant betas (beyond 1% point) are printed in boldface type.

^b Numbers in parentheses refer to numbers of varieties and selections.

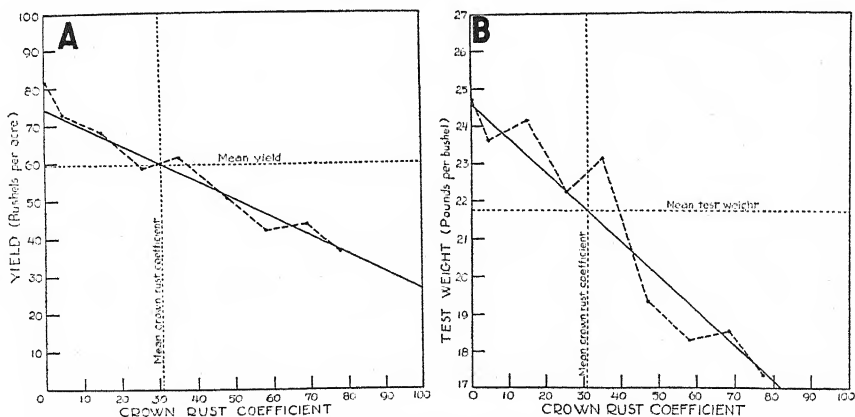


FIG. 1. A. Simple regression of yield on coefficient of crown rust in 159 varieties and selections of oats grown at Kanawha, Iowa, in 1938. B. Simple regression of test weight on same coefficient.

The simple regressions of yield on crown rust for the different groups were as follows:

$$\begin{array}{ll} (80) \bar{Y} = -.45C + 65.55 & (78) \bar{Y} = -.47C + 71.47 \\ (125) \bar{Y} = -.40C + 74.49 & (159) \bar{Y} = -.47C + 74.29 \\ (442) \bar{Y} = -.44C + 72.16 \end{array}$$

Thus, considering crown rust alone, for each unit increase in coefficient of crown-rust infection the yield of the 442 varieties and selections was decreased an average of 0.44 bushel per acre; and with a coefficient of 100 the average decrease caused by crown rust alone averaged 44 bushels per acre. At Kanawha the yield of the 159 varieties and selections was decreased an average of 0.47 bushels per acre for each unit increase in coefficient of crown rust. This relationship is illustrated in figure 1, A.

The partial regression equations for estimating test weight were as follows:

$$\begin{array}{ll} (80) \bar{W} = -.07C + .01L + .06H - .05D + .16Y + 19.91 \\ (78) \bar{W} = -.01C - .03L + .19H + .40D + .16Y + 4.05 \\ (125) \bar{W} = .00C - .02L + .21H + .01D + .14Y + 6.64 \\ (159) \bar{W} = -.03C - .03L + .17H - .08D + .12Y + 11.60 \\ (442) \bar{W} = -.02C - .01L + .09H + .03D + .20Y + 7.08 \end{array}$$

Test weight was related more closely to yield than to any of the other characters observed. In the presence of the other variables, crown rust did not have a highly significant effect on test weight in the rod-row tests at Ames. For each unit increase in coefficient of crown rust in the 80 varieties and selections at Ames and 159 at Kanawha the test weight decreased an average of 0.07 and 0.03 pound per bushel, respectively. The simple regressions of test weight on crown rust for the different groups were as follows:

$$\begin{array}{ll} (80) \bar{W} = -.14C + 24.26 & (78) \bar{W} = -.09C + 26.11 \\ (125) \bar{W} = -.06C + 24.71 & (159) \bar{W} = -.09C + 24.60 \\ (442) \bar{W} = -.07C + 25.89 \end{array}$$

The relationship between the average test weight and average coefficient of crown-rust infection for the 159 varieties and selections grown at Kanawha is illustrated in figure 1, B. The selections from the cross of Markton × Rainbow tended to have slightly higher test weights than selections from other crosses, when crown rust was held constant.

SUMMARY

A severe epiphytotic of crown rust of oats occurred at Ames and Kanawha, Iowa, in 1938. Coefficient of crown-rust infection (percentage of infection × type) showed a higher negative correlation with yield and test weight in 1938 than did either percentage of infection or type of infection alone, indicating that coefficient of infection is a slightly better measure.

Coefficient of crown-rust infection showed a high negative correlation with yield and test weight in all tests at Ames and Kanawha in 1938. The

total correlations between coefficient of crown rust and yield were all highly significant and ranged from $-.75$ to $-.80$.

For each unit increase in coefficient of crown-rust infection (in the presence of the effect of test weight, date ripe, height, and lodging) yield was decreased an average of 0.21 to 0.32 bushel per acre. For each unit increase in the crown-rust coefficient alone, yield was decreased an average of 0.40 to 0.47 bushel per acre.

Yield and test weight were highly correlated in all tests. With each pound increase in test weight, yield was increased an average of 0.47 to 2.39 bushels per acre.

Lodging, height, and date ripe were all negatively correlated with yield, i.e., for 1938 the stiffer-strawed, shorter, and earlier varieties tended to be higher-yielding, on an average, than the weaker-strawed, taller, and later varieties.

Breeding for resistance to crown rust is of paramount importance when conditions such as those in Iowa in 1938 are encountered. Similar damage from crown rust occurred in 1927 and 1935. Such frequent occurrence of epiphytotics in Iowa emphasizes the great need for and potential value of Boone, Marion, and other new varieties with resistance to crown rust, stem rust, loose smut, and covered smut.

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CLASSIFICATION AND NOMENCLATURE OF TOBACCO VIRUSES¹

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The viroses of tobacco and the viruses causing them have undoubtedly received more detailed study than any other similar group. They are, therefore, in a better position to be classified than many other less-known virus groups. Unfortunately, previous attempts to classify viruses have been more concerned with nomenclature than with a satisfactory grouping of the viruses themselves. As a result the tobacco viruses have not been satisfactorily classified. It is difficult to separate completely a discussion of classification from that of nomenclature. The present paper, while primarily concerned with classification of the viruses causing diseases in tobacco, necessarily considers nomenclature also. For those who do not wish to accept the system of nomenclature used, the classification can be considered independently.

Two proposed systems of virus nomenclature, which have received most attention, are those of James Johnson (5) and K. M. Smith (7). They are identical in principle, but the genus name of a host has been substituted in the latter for the common name in the former. Smith's publication evidently is based on unpublished material submitted to him by Johnson, as it contains many virus numbers credited to Johnson that had not previously been published. Both systems have the objection that classification based on natural relationships has been disregarded. Strains of a virus and distinctly different viruses are given coordinate rank. For example, *Lycopersicum* virus 2 is a strain of the tobacco-mosaic virus collected on tobacco in Kentucky, yet it is given the same rank as the tobacco-mosaic virus *Nicotiana* virus 1 and considered to be as distinct an entity as the tomato-spotted-wilt virus *Lycopersicum* virus 3; and tobacco viruses 1, 2, 6, 7, and 9 are all very evidently strains of the tobacco-mosaic virus (5). More recently, Holmes (2) has proposed a latinized binomial-trinomial system. Although he has attempted to make natural groups of the viruses treated, he has not made full use of the knowledge available with respect to natural relationships where they occur and has not separated distinctly different viruses when the evidence clearly indicated that they might be separated. Perhaps too much attention has been given to plant symptoms in grouping rather than to the virus itself, which is the entity being classified. Nevertheless, the proposal eliminates the numerous objections that can be raised to the numbering systems and gives to the biologist a system of nomenclature with which he is familiar; and one adaptable to continuous revision, as occasions arise.

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Any system of classification and nomenclature, to have international standing and to withstand the test of time, must be based as far as possible on what appear to be natural relationships. It seems that many of the plant viruses can now be placed in what may be termed natural groups. The proposals of Smith and Holmes have included all of the better-known plant viruses. Obviously, neither was in a position to know more than a few of the viruses at all intimately, so that a revision of classification of special groups of viruses is in order. This paper presents a grouping of the tobacco viruses, which is the result of a study of those commonly found in tobacco fields in Kentucky over the past 20 years and also includes viruses causing disease yearly in other parts of the world. It includes viruses that necessarily will be dealt with by specialists in other fields, but the experiences gained in a study of these viruses in tobacco may be of value to them. The names proposed by Holmes will be followed where his terminology can be used; where it cannot be used, a new name will be suggested. The characteristics of the viruses will not be repeated in this paper, as these are given by Holmes in a condensed form.

Until more is known about interrelationships of strains of the viruses, little attention should be given them from the standpoint of an international system of classification and nomenclature unless a particular strain can be shown to be well-established in nature and to differ markedly from the ordinary mutant strains of the virus. Strains should be studied in detail and given laboratory designations, rather than ones for an international system. The confusion likely to arise if strains are given names in an international system is well exemplified by designations given to the *Nicotiana* viruses by Smith. It is doubtful if *Nicotiana* viruses 1, 1A, 1B, 1C, 1D, 2, 3, 4, 9, 12A, 12B, 13, 14, and 15 represent entities well-established in nature that other workers are likely to find and identify, with any degree of certainty, with the original collections; whereas *Nicotiana* virus 1 (if taken to represent the large number of tobacco-mosaic-virus strains), *Nicotiana* viruses 7, 8, 10, 11, 12 (with its several strains) represent distinct kinds of viruses that can be found each year and identified with a high degree of certainty.

The viruses here presented for consideration are the following: Tobacco-mosaic, Cucumber-mosaic, Veinbanding, Tobacco-ring-spot, Etch, Tobacco-streak, Tomato-spotted-wilt, Tobacco-leaf-curl.

A simple key has been prepared for identification of these viruses, if found in tobacco, which gives some idea of the basis upon which identification is made. The key could be made much more complicated, but is left in this simple form for the convenience of those not familiar with the tobacco viruses.

KEY TO THE VIRUSES OF TOBACCO THAT ARE FOUND YEARLY
CAUSING DISEASE IN COMMERCIAL PLANTINGS

1. Infectivity not destroyed by drying.

Tobacco-mosaic virus. *Musivum tabaci*.²

² The names used in this key will be discussed later in this paper.

2. Infectivity destroyed by drying.

1¹ Transmissible to tobacco by plant sap.1² No infection on cucumbers.1³ Local necrotic lesions on *Nicotiana tabacum*.1⁴ Local necrotic rings or arcs on rubbed leaves of *N. tabacum* and fine necrotic etching on invaded leaves. Tobacco-etch virus. *Foliopellis erodens*.2⁴ Large local necrotic lesions on leaves of *N. tabacum* and systemic streak with crooking of stalks. Tomato-spotted-wilt virus. *Lethum australiense*.2³ No local lesions on rubbed leaves of *N. tabacum*. Veinbanding systemic symptoms. Veinbanding virus. *Murialba venataenia*.2² Infection on cucumbers.1³ Mosaic in tomato. Cucumber-mosaic virus. *Murialba cucumeris*.2³ No infection, or ring patterns on tomato. Tobacco-ring-spot virus. *Annulus tabaci*.2¹ Transmitted to tobacco with difficulty, if at all, by plant sap.1³ Severe streak and stunting in *N. tabacum*, followed by more normal growth; sap transmission during early necrotic stage. Tobacco-streak virus. *Tractus orae*.2³ Leaves of *N. tabacum* severely rugose and curled. Transmission by *Bemisia gossypiperda*. Tobacco-curl virus. *Ruga tabaci*.

Holmes' genus *Marmor* evidently includes several viruses that are distinct and should be given generic rank. The tobacco-mosaic virus has not been shown related in any way to many of the other species in the genus *Marmor*. (See discussion of *Marmor*, page 827.)

Musivum³ (mosaic), new genus

This genus is characterized by the ability of the virus to withstand drying, by high thermal inactivation of about 90° C. for 10 minutes, and by serological relationships with the type that is the common tobacco-mosaic virus.

Musivum tabaci, new comb.**Tobacco-mosaic Virus**

Synonyms. *Protobios mosaicus* var. *tabaci* d' Herelle, *Marmor tabaci* Holmes, Tobacco viruses 1, 2, 6, 7, 9; *Nicotiana* viruses 1, 1A, 1B, 1C, 1D, 2, 6, 9; *Lycopersicum* viruses 1, 1A, 2; Rotterdam B virus; Tomato aucubamosaic virus; masked-symptom virus and the viruses of ring mosaics, white mosaics, yellow mosaics; and of numerous other terms applied to the mosaic disease.

It should be recognized that the virus is extremely variable and that numerous strains can be isolated from what appears to be a pure strain. The same process has been going on in nature as evidenced by the numerous strains of the virus already described. The common field mosaic, as it occurs in tobacco, is made up of numerous strains, including those with all degrees

³ Suggested to the writer by Dr. H. H. McKinney.

of distortion; all degrees of color from pure-white to dark-green patterns; all degrees of burning from non-burning strains to those that cause extensive necrosis in field plants; and strains causing all degrees of local spotting, including no apparent symptoms, varying degrees of chlorosis, and varying degrees of necrosis on certain genotypes of *Nicotiana tabacum*, from pin-point necrotic spots to necrotic spots apparently identical with those produced by the numerous strains on *Nicotiana glutinosa*. The varietal name *vulgare* applied by Holmes to the field mosaic virus necessarily includes the majority of these strains. Each of the varietal names *aucuba*, *deformans*, *canadense*, *lethale*, *obscurum*, *immobilis*, and *artum* describes a symptom that may be produced by other strains of the virus, that may, however, differ significantly from the strain to which the name has been applied. Therefore, the uninitiated might collect a virus that withstood drying and produced the *aucuba* patterns on tomato and tobacco and had the ability to produce necrotic spots on *Nicotiana sylvestris* and certain tobacco varieties and still be distinct from var. *aucuba*. Such a one is the white mosaic virus obtained from pepper in Kentucky by E. M. Johnson (3). Confusion will be avoided if the numerous strains of the tobacco-mosaic virus collected and studied are given laboratory designations, for at present there is no method of describing a strain in a manner sufficiently clear that a similar strain can be identified with it. The latinized varietal names of this virus proposed by Holmes should, therefore, be disregarded, but the common names of strains retained to be applied only to the original collection and its identical progenies.

The English cucumber-mosaic viruses, which withstand drying, have been shown to be serologically related to the tobacco-mosaic virus, although their host range is entirely different. The name *Musivum astrictum* would indicate this relationship to the tobacco-mosaic virus, and yet set the virus off as a distinct species. This new combination is proposed. Already 2 strains have been described but should be given only laboratory designations for the present.

McKinney's mild dark-green mosaic from *Nicotiana glauca* (6), which does not cause infection in tomato, is obviously different in this respect from the numerous strains of *Musivum tabaci* and might well be considered a subspecies of *Musivum tabaci* or even a distinct species.

Murialba (Muria-pickle, Alba-white) new genus

This virus is serologically unrelated to *Musivum*, does not withstand drying, has a thermal death point of about 74° C. in 10 minutes, and either causes mosaic in cucumbers or is serologically related to the virus of common cucumber mosaic. The virus is commonly transmitted by species of *Aphis*.

Murialba cucumeris, new comb.

Cucumber-mosaic Virus

Probable synonyms: *Marmor cucumeris* and varieties, Cucumber virus 1, Cucumis viruses 1, 1A, 1B, 1C; *Nicotiana* viruses 3 and 4; Tobacco viruses

3, 8; Delphinium viruses 1 and 2; spinach-blight virus, southern celery virus, lily-mosaic virus, tulip-break virus in part.

Studies of the virus as it occurs in tobacco indicate that there are at least 10 strains that are easily distinguishable from one another in tobacco and with less ease in cucumbers. The genus is evidently a variable one and it may be expected that numerous strains will be found to be causing mosaic in cucumbers, celery, and other susceptible species. For the time being it would seem better to consider them all as mutant strains of *Murialba cucumeris* until the numerous strains can be more carefully studied. It is to be anticipated that viruses having entirely different host ranges than the more common strains of the cucumber virus will be found that are related serologically to the cucumber-mosaic virus and might logically be considered as distinct species of this genus.

Murialba venataenia, new species

Veinbanding Virus

Synonyms: *Marmor cucumeris* var. *upsilon*. Vein border, Tobacco virus 4 in part; Solanum virus 2; Potato Y virus.

The virus probably is best known as the cause of mottling in many potato varieties. It is commonly found in tobacco growing in the vicinity of potatoes and its presence in tobacco is almost certain evidence that potatoes have been growing in the vicinity of the tobacco at some time during the growing season. Chester (1) has claimed that the virus is serologically related to the cucumber-mosaic viruses, on the basis of tests made with viruses given to him as pure strains. The cucumber-mosaic and veinbanding viruses are commonly found associated in the same tobacco plant in the field, and the presence of the veinbanding virus is not easily detected in tobacco infected by both viruses, in the greenhouse. There is no evidence that one protects against the other. The veinbanding virus has characteristics indicating that it is distinct from the strains of the cucumber-mosaic virus.

If veinbanding and cucumber-mosaic viruses are proved to be serologically related then the veinbanding virus should be considered a distinct species of the cucumber-mosaic genus *Murialba* coordinate with the species *cucumeris*. If the serological relationship does not exist, it should be elevated to a generic rank. Until proof is given that a serological relationship does not exist, the name *Murialba venataenia* (vena-vein, taenia-border) is proposed.

Annulus tabaci Holmes

Tobacco Ring-spot Virus

Synonyms: Hieroglyphics virus, Nicotiana viruses 12, 12A, 12B, green ring-spot virus, yellow ring-spot virus.

The ring-spot virus is obviously distinct from the tobacco- and cucumber-mosaic viruses, and deserves the generic rank given it by Holmes. It is doubtful, however, whether the family, Annulaceae, should be recognized

on the basis of supposed recovery from the disease. The plants do not recover but simply pass from the ring-spot stage to a stage without ring patterns, but frequently with severe leaf-tip and edge chlorosis and necrosis (produced at about 20° C.), or prominent chlorosis of otherwise normal leaves. Under optimum conditions growth may be almost normal. The change in symptoms occurs when embryonic tissue is finally invaded. Ring patterns are an indication of invasion of mature cells. Ring-spot patterns may be initiated on so-called recovered plants by cutting the plant back to a dormant, uninvaded bud the shoot from which will develop ring patterns when it finally becomes invaded. Pollen sterility, either partial or complete, occurs and is further proof that recovery from this virosis does not occur. Seed transmission is common, but is sometimes difficult to demonstrate with the non-yellowing strains. Three collections have been carefully studied and each has been found somewhat different from the others and has been given a varietal name by Holmes. The writer has evidence that if further collections are carefully studied each will be found to differ sufficiently from the 3 already named to warrant recognition. Here again it would seem wiser to disregard the latinized varietal names proposed by Holmes and designate collections of this virus by laboratory names only.

Foliopellis (folio-leaf, pellis-hide), new genus

The type virus is the cause of the etch disease of tobacco on the Experiment Station farm at Lexington, Ky., and elsewhere in the United States. It is not related serologically to *Musivum*, *Murialba*, and *Annulus*; has a known host range limited to the Solanaceae, and is transmitted by species of *Aphis*. The name, *Foliopellis*, refers to a leather-like appearance of affected leaves at a certain stage of the disease.

Foliopellis erodens, new comb.

Etch Virus

Synonyms: *Marmor erodens* Holmes. Etch + virus, severe-etch virus, tomato-etch virus, *Datura-Z* virus, *Nicotiana* virus 7.

In addition to the characteristics given by Holmes, this virus is transmitted by aphids.⁴ It has been collected in Kentucky, Ohio, Georgia, New York and New Jersey. In Kentucky it appears to be limited to areas near old garden sites or to tobacco, pepper, or tomato growing in vegetable-gardening areas. It is readily transmitted to virus-free seedling potato plants, where it persists for several tuber generations and suggests that it may have been a common virus of some now obsolete potato variety. The virus persists in solanaceous perennial weeds. Three strains have been recognized, to 2 of which Holmes has applied a trinomial. There is no good reason for believing that any new collection could be identified absolutely with any of the 3 original strain collections. Recognition that strains

⁴ Unpublished evidence obtained by the writer and E. M. Johnson and confirmed by A. A. Granovsky to whom the virus was sent.

of this virus occur seems all that is warranted at present and the varietal names should not be recognized.

Tractus (streak), new genus

The virus of tobacco streak is obviously distinct from the tobacco-ring-spot virus in thermal inactivation, in not being readily transmissible from mature tissue, except by grafting, in host range, and in symptoms produced. Mottling occurs in tobacco, following the severe "shock" stage, and is the only symptom produced in other species, so that "recovery" cannot be considered as a characteristic. The virus gives no protection against the other genera discussed and is obviously distinct. Therefore, the virus should be given generic rank. The name *Tractus* is proposed. The virus is the cause of a streak in *Nicotiana tabacum* in areas where sweetclover is grown. It is characterized by thermal inactivation at about 53° C. in 10 minutes, infectivity is lost in about 30 hours at room temperature. It is insect-transmitted, but the vector is not known.

Tractus orae, new comb.

Tobacco-streak Virus

Probable synonyms: *Annulus orae*, Streifen- und Krausel-krankheit, Streak, Tobacco streak, *Nicotiana* viruses 5 and 8, and possibly *Marmor vastans*, the potato yellow-dwarf virus, *Solanum* virus 16.

The streak virus, the virus of potato yellow-dwarf, and the diseases they cause have much in common. The writer has transmitted the streak virus to tomato by grafting where it causes a necrotic spot of a few leaves but little or no stem necrosis, and then makes apparently normal growth. He has, by grafting, caused streaking in seedling-potato stalks and in Cobbler plants to which affected tobacco scions had been grafted. Thus, 2 supposed differences between the streak and yellow-dwarf viruses have been eliminated. The yellow-dwarf virus does not cause severe streaking in Burley tobacco and differs in this way from streak.⁵ There is fairly good evidence that the tobacco-streak virus originates in sweetclover growing adjacent to the affected tobacco, while the yellow-dwarf virus is found in red clover. If the tobacco-streak virus is not identical with the yellow-dwarf virus it is probably closely related.

Lethum australiense Holmes

Tomato-spotted-wilt Virus

Probable synonyms: Corcovo virus, Faucett, 1921, Soriano, 1931, Kromnek or Kat River virus, *Lycopersicum* virus 3.

This virus appears to be different from any of those previously described. It has not been identified on tobacco in Kentucky or anywhere else in the United States so far as the writer is aware. If it proves to be a variable virus there would seem to be no reason for naming each strain with a trinomial, as Holmes has done with 2 strains.

⁵ The yellow-dwarf virus used was kindly furnished by Dr. L. M. Black.

Ruga tabaci Holmes

Tobacco-leaf-curl Virus

Synonyms: Tobacco-leaf-curl virus, tobacco-cabbaging or crinkle virus, tobacco kroepoek virus, tobacco-crinkly-dwarf virus, tobacco-gila virus, Nicotiana virus 10.

Little is known of this virus, except that it is spread by the white fly, *Bemisia gossypiperda*, and that there are at least 4 strains varying in severity of resultant curling. It has a moderately wide host range. It has not been discovered in the United States.

DISCUSSION

The writer has attempted to classify the viruses known to cause field diseases of tobacco year after year into 8 easily recognized genera. The proposal differs in no fundamental way from the virus groups proposed by Valteau and Johnson at the Nashville meeting in 1928 (8) and elaborated by E. M. Johnson in 1930 (3). The tobacco viruses have been more thoroughly studied perhaps than any other group and are in a better position to be classified than perhaps any other group. Whether it will be possible to treat other groups of viruses in like manner, until more is known of their relationships, is open to question. Obviously, all viruses that can cause disease in tobacco have not been included. Of the 8 genera discussed only 6 are known to cause a field disease of tobacco in the United States. The system of nomenclature proposed by Holmes has been followed as it seems to be the least objectionable of those proposed and is a system with which biologists are already familiar. Holmes' use of trinomials for the mutant strains of viruses has not been followed because it is believed to be impossible at the present time to define any strain of a virus so that it can be recognized in the future; but the genera and species here proposed can be recognized accurately with a limited amount of study. One problem in classification and nomenclature of viruses will be that of seeking relationships among apparently unrelated viruses and grouping them. Viruses that are proved to be related and yet differ from one another to a greater extent than strains ordinarily do would have relationship shown through a common genus and differences by being separated as species. For example, the English cucumber mosaic virus withstands long periods of drying and, therefore, is similar to the tobacco-mosaic virus. Serological studies indicate that it is related to the tobacco-mosaic virus. The two could, therefore, be considered as coordinate species of a common genus. As other such relationships are discovered, classification can proceed on a logical basis.

For those viruses that appear to be found on only a single host and in which relationships are not known and where there are no well-defined characters to set them off as belonging to a distinct genus, the writer proposes some "catch all" genus in which these viruses could be placed. The majority of the viruses in Holmes' genus *Marmor* are of this class, as are

many of those listed by Holmes in supplement III "Viruses not treated in this handbook." If the proposals in the present paper are accepted, then the genus *Marmor* could be reserved for this purpose with but little confusion. As Holmes has made no attempt to define the genus *Marmor* it might be defined as including viruses causing diseases usually characterized by persistent mottling, or necrotic spotting on some genotypes, which have not been sufficiently studied so that their relationships to other recognized viruses are known.

If relationships were later found with a species of a known genus the virus would either be considered a strain of a recognized species or, if it differed sufficiently, a species of the genus to which it was found to be related. Whenever it could be demonstrated that viruses in the genus *Marmor* were unrelated to any of the accepted genera they could be raised to generic rank.

At present there are 2 known methods of determining relationships between viruses other than by comparing symptoms and physical reactions of the viruses. These are the serological reaction and the protection or so-called immunity reaction. The serological method will probably prove to be an accurate means of determining relationships in viruses, as it has in plants and animals, if it is clearly demonstrated that only a single virus was used as antigen. By the serological technique it may eventually be possible to prove relationships between viruses that have no antigens in common. For example, one virus may contain the antigens A, B, and C, another the antigens C, D, and E, and another E, F, and G. The first virus and the last would show no relationship, but the first and second would be related through the common antigen C, and the second and third through the common antigen E. Therefore, the first and third might be considered as distantly related. It would seem that relations of this type might suffice to define species of a common genus.

The so-called immunity reaction or, better, the protective effect of one virus against another strain of the same virus, has been used to some extent in classifying viruses. It undoubtedly has merit if properly used, but, if improperly used, will lead to further confusion. For example: If a tobacco plant, already bearing several well-developed leaves, is inoculated with a green strain of tobacco-mosaic virus and left until the growing-point leaves show mosaic, typical of the virus strain used, and is then inoculated with a rapidly multiplying white strain on the mottled leaves, the white strain is not likely to become established sufficiently to become evident in the new leaves as they develop. This is considered as evidence that the first virus has protected the plant against the second virus and that the 2 viruses are related. If, however, the white strain be inoculated into a virus-free leaf, already well-developed when the first inoculation was made, the white strain will find no competition with the green and will multiply as though in a healthy plant. The white-mosaic virus will then pass from this leaf up to the developing leaves of the growing point, which are only partially invaded by the first virus, will become established there, develop symptoms typical

of the white-mosaic virus, and either persist with the green, virtually replace it, or be finally replaced by it, depending upon rate of multiplication and distribution of the two viruses (4). If the white and green strains both persist, or if the white replaces the green, neither protection nor immunity has been demonstrated and the viruses would be considered unrelated. Thus, by choice of 1 of 2 protective techniques, now in common use, one may demonstrate either that two viruses are related or that they are distinct. The explanation of this paradox is simple. In the first case a solidly invaded leaf is inoculated with another strain of the same virus. The diseased cells, already thoroughly invaded by the first virus, do not make a favorable medium in which the second can multiply rapidly, spread to adjoining cells, and make its presence evident (but it has not been demonstrated that the second virus, even under these unfavorable conditions, may not enter some cells and become established). In the second instance where a lower uninjured leaf is inoculated by the second virus there is nothing to prevent its multiplication, release, and long-distance carriage to growing-point leaves that are either uninjured or only partially injured by the first virus. The two viruses entering different groups of healthy cells have an equal chance of survival if they multiply at the same rate. Viruses that invade embryonic tissue solidly, or nearly so, are more apt to give complete protection against related strains than viruses that invade young leaves more slowly and produce mottle patterns, ring-spot patterns, or specking, especially if the grafting method is used. In spite of these objections to the protective reaction it will undoubtedly prove of value, if properly used and interpreted, in classifying certain types of viruses.

SUMMARY

Eight viruses causing disease in commercial plantings of tobacco are discussed. Viruses that show no relationship to others are given generic rank.

The following new genera are proposed: *Musivum*, tobacco-mosaic virus; *Foliopellis*, etch virus; *Murialba*, cucumber-mosaic virus; and *Tractus*, streak virus. The veinbanding virus is given specific rank, coordinate with the cucumber-mosaic virus. The streak virus is removed from Holmes' genus *Annulus* and given generic rank. The family Annulaceae of Holmes is not recognized because based on supposed recovery that does not occur. Strains of viruses, when obviously mutants, should be recognized by laboratory designations rather than by trinomials. The use of serology and protection as means of classifying viruses are discussed.

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PHYTOPHTHORA INFESTANS ON TOMATO

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INTRODUCTION

Montagne's description of *Phytophthora infestans* from potato was closely followed by Payen (15) with a description of the same fungus from tomato. Inoculation of potato tubers with the fungus from potato, and from tomato, resulted in similar fungous growths and disease symptoms (16).

Since these early accounts by Payen, late blight of tomato has been reported from practically every tomato-growing region of the world and has become one of the major diseases of the crop.

Early observers did not question the passage of the fungus from potato to tomato. Thaxter (26), Clinton (5), Sturgis (24), Smith (23), and others refer to the disease as caused by the potato fungus. McAlpine (11, 12, 13) obtained infection on green tomato fruits by the use of sporangia from potato, and on potato tubers with sporangia from tomato. He also noted the spread of blight from potato foliage to adjoining tomato fruits (13).

In spite of apparently indiscriminate passage of the fungus from potato to tomato, Reed (19) suggested that physiologic specialization might exist. He observed that while tomato blight was severe in Virginia, with little potato blight, the contrary was true in New England. Later, Reed (20) noted a uniform spread of blight on tomatoes and potatoes and concluded that early-planted potatoes in Virginia largely escaped infection. Melhus (14) attributed contradictory statements regarding the species of *Phytophthora* on these two plants to the fact that the fungus spreads and fruits more sparingly on tomato foliage than on potato.

Investigations by Giddings and Berg (9), and Wiltshire (27) showed that the fungus from potato did not cause typical blight of tomato foliage. Berg (1), in a comprehensive investigation, proved that tomato strains of *Phytophthora infestans* from West Virginia and Australia were much more virulent in their attack on tomato foliage than were a large number of potato strains of widely separated origin. Recent investigations by Röder (21) in Germany and Small (22) in England have substantiated Berg's conclusion that the fungus attacking tomato is a physiologic race different from that on potato. Röder has called the tomato race the "T biotype."

Although cross-inoculations show that the fungus from potato does not cause typical tomato-foilage blight, several contradictions are, nevertheless, presented.

1. If tomato blight is not associated with potato blight in some manner, why is there almost unanimity in field observations that tomato blight is more serious in the neighborhood of diseased potatoes? Evidence by Berg (1), Clinton (6), Fromme and Thomas (8), McAlpine (13), Payen (16), Reed (20), Small (22), and Taubenhau and Ezekiel (25) expresses this view.

2. If tomato blight does not originate with potatoes, why should tomato blight almost invariably appear several days to weeks later on tomato than the first blight on potato? This phenomenon has been observed repeatedly (1, 2, 6, 7, 10, 13, 22, 27). In this connection it is interesting to note McAlpine's record (11, 13) that *Phytophthora infestans* was first recorded in Australia on potatoes in 1909 and that it was first observed there on tomatoes in 1910. Taubenhau and Ezekiel (25) reported that late blight, previously unimportant in Texas, was severe in 1931 on potatoes and tomatoes. They assume that the fungus, which was observed to spread from the potatoes to the tomatoes, was introduced from the North in the potato seed. That the introduced fungus was the tomato strain appears highly improbable, since that strain is so rare in the North. Röder (21) isolated 66 cultures of *P. infestans* from potato, and not one was the tomato strain.

3. How does the tomato strain overwinter? Clinton (6) concluded that blighted potato tubers must be the source of primary inoculum, because oospores could not be found under a great variety of conditions; the fungus did not survive the winter in any kind of tomato tissue or in soil that had supported blighted tomatoes the previous year; no alternate host could be found; and *Phytophthora thalictri*, rarely found in Connecticut, could not infect tomato. Reed (20) was unable to demonstrate overwintering of *P. infestans* in infected tomato seed. Although Boyd (2, 3, 4) showed that the fungus may survive in mild winters in infected seeds and vines in Massachusetts, this must be a rare occurrence, since he (4) says that late blight of tomatoes was unimportant in that State between 1905 and 1932.

OBJECT OF EXPERIMENTS

The object of the following experiments was to make a study of the possible effects of host on virulence of *Phytophthora infestans* and the relation of such virulence changes, if any, to the above seemingly contradictory statements concerning blight of potatoes and tomatoes. Although Berg (1) and Röder (21), considering the possibility of adaptation to the particular host, found that the potato and tomato strains were completely stable when cultured on tomato fruits and potato tubers, respectively, neither investigator attempted serial passages of the potato strain through tomato foliage. Inasmuch as it is possible to increase the virulence of *P. infestans* for certain potato hybrids by passing the fungus through resistant potato plants (18),

it appeared highly desirable to make serial-passage experiments with the potato through tomato foliage.

MATERIALS AND METHODS

Two cultures of *Phytophthora infestans* were isolated from blighted potatoes from widely separated regions in New York State. A number of single-swarmspore isolations from these cultures appeared identical in pathogenicity and in other respects, so that two of them, one from each original culture, were arbitrarily saved. A tomato culture was isolated from a diseased fruit found in a field of severely blighted tomatoes. All cultures were maintained in the laboratory on raw potato slices.

The tomato variety Bonney Best was used for serial-passage experiments. Several other varieties, including Marglobe, San Marzano, Allred, and Red Pear, were used in routine tests of cultures. All varieties showed typical resistance to the potato cultures and susceptibility to the tomato cultures. The Green Mountain variety of potato was used in experiments requiring potato foliage or tubers.

Plants, inoculated by atomizing with a suspension of swarmspores, or by placing drops of a suspension on the foliage with a pipette, were placed in a moist chamber for several hours. The infected plants were returned to the moist chamber for fungus sporulation. Sporangia were collected, germinated at 12° C., and the swarmspores reapplied to tomato foliage. The effect of passage of the potato cultures through tomato foliage was determined by comparative inoculations on tomato foliage with the potato culture, taken directly from potato leaves or tubers, and with the tomato culture taken from tomato leaves. Virulence for tomato was determined by length of incubation period, the presence or absence of stem, petiole and tip-leaf lesions, the amount of sporulation, and whether the infected plants died or recovered.

In preliminary experiments, 4 tomato plants were inoculated at each fungus transfer. In later experiments only 1 or 2 plants were inoculated at each transfer.

EXPERIMENTAL RESULTS

Behavior of Cultures on Tomato

Potato Cultures. The two potato cultures were identical in their behavior on tomato. Symptoms were similar to those described by Berg, Röder, and others. Tiny, brown streaks, which appeared on leaves 66 to 72 hours after inoculation, when held at about 21° C., quickly changed to glazed, oily spots. Lesions on lower leaves occasionally spread throughout the leaflet, but, ordinarily, the fungus died within 4 or 5 days after the lesion appeared. Usually no petiole or stem infection developed. Small, superficial lesions, which rarely appeared, never enlarged.

Young apical leaves were exceedingly resistant. If they became infected at all, the small, black, sharply delimited lesions failed to enlarge.

Slight sporulation was visible on lesions on older leaves after a period of 24 hours in a saturated atmosphere. No sporulation occurred on the young leaf or petiole lesions. When many lesions were formed on the older, lower leaves, abscission followed, so that 2 or 3 weeks after inoculation, the plants appeared entirely healthy.

Tomato Culture. Symptoms produced on tomato plants by the tomato culture were essentially the same as those described by Berg, Röder, and others. Distinct lesions, evident within 55 to 60 hours after inoculation, appeared at approximately the same time on all leaves, stems, and petioles. Profuse sporangial formation occurred within 12 to 15 hours in a saturated atmosphere. Sporulation was nearly as heavy on lesions on tip leaves and petioles as on mature leaves.

Effect of Passage of the Potato Culture Through Tomato Foliage

Experiments in serial passage of the potato culture through tomato foliage were conducted over a period of about 18 months. It was found that passage of the potato culture of *Phytophthora infestans* through tomato foliage gradually increased the virulence of the fungus until a culture was obtained that was indistinguishable from the natural tomato culture. The experiment was repeated 6 times, using both potato cultures, the fungus being passed through tomato foliage from 7 to 22 times in the different experiments.

In the first experiment 4 tomato plants were inoculated with the potato culture. As rapidly as possible, the fungus was then transferred from tomato to tomato for a series of 15 transfers during a period of 87 days. At each of the first 10 transfers the culture was compared with the potato culture on tomato, and other comparisons were made at the 12th and 15th transfers.

Very little difference in virulence could be observed between any two consecutive transfers on tomato, except that sporulation on plants infected with T(1) culture (potato culture passed once through tomato) was noticeably heavier than on a plant infected simultaneously with the potato culture taken from potato tubers. The virulence increase was very gradual and progressive up to and including the 7th passage. From the 8th to the 15th transfers, no further increase of virulence could be observed. After 7 passages through tomato, the incubation period was from 18 to 24 hours shorter than for plants infected with the potato culture taken from potato. Sporulation was profuse and infection occurred on petioles, stems, and apical leaves.

In order to make a simultaneous comparison of cultures after various numbers of passages through tomato foliage, the following scheme of inoculations was carried out: A tomato plant was inoculated with the potato culture and then the fungus was passed 7 times through tomato, transfers being made at approximately weekly intervals. At the 3rd and 5th transfers, other tomatoes were inoculated with the potato culture. All were then trans-

ferred simultaneously until cultures T(2), T(4), and T(7) were at hand. At this time the potato strain, cultures T(2), T(4), T(7), and the natural tomato culture were compared on tomato plants. All plants were of the same age and all subjected to identical conditions. Inoculations were made by atomizing with an approximately equal number of swarmspores of each culture.

The results of the tests of various cultures, summarized in table 1, again emphasize the gradual and progressive nature of the virulence increase. The T(7) culture was fully as pathogenic on tomato as was the natural tomato culture.

TABLE 1.—*Effect on virulence of Phytophthora infestans by passage of a potato culture through tomato foliage*

Culture	Incubation period	Tip-leaf lesions	Stem, petiole lesions	Sporulation ^a		Condition of plant 15 days after inoculation
				Old leaves	Tip leaves	
Potato	Hr. 66	Tiny specks, no increase	None	+	0	Lower leaves dropped; few dry lesions of 8–10 mm.
T(2)	62	Tiny specks, no increase	Few black specks, no increase	++	+	Lower leaves dropped; few dry lesions of 8–10 mm.
T(4)	58	Larger than T(2)	Lesions enlarged considerably; petiole not consumed	+++	++	All infected leaves dropped; stem infection insufficient to kill
T(7)	58	Nearly the same as old leaves	Petioles and succulent stems consumed	++++	+++	Dying, stem wilted
Tomato	58	Nearly the same as old leaves	Petioles and succulent stems consumed	++++	+++	Dying, stem wilted

^a 0, no sporulation; +, few isolated sporangiophores; ++, readily visible; +++, abundant; +++++, abundant; leaves white.

The culture mentioned above, carried 7 transfers on tomato foliage, was then maintained on tomato foliage for a total of 22 passages. No detectable change occurred between the 7th and 22nd passages.

Virulent Potato Culture on Tomato Foliage

Reddick and Mills (18) showed that a culture of *Phytophthora infestans*, unable to infect certain potato hybrids, attacked those hybrids readily after the fungus had passed through certain resistant varieties. The question immediately arose as to how tomato plants would react to this culture of increased virulence. Four tomato plants were inoculated with the virulent culture and 4 others with the ordinary potato culture. The 2 cultures pro-

duced similar symptoms on tomato foliage, showing that the increase in virulence for the potato hybrids had not increased the virulence for tomato. This culture was then passed 9 times through tomato foliage. Virulence for tomato was gradually increased until the 6th or 7th passage, when it appeared as virulent for tomato as did the natural tomato culture. It is interesting to note that during the 52 days that the fungus was maintained on tomato foliage, full virulence was retained for the potato hybrids.

Comparisons of Tomato and Potato Cultures on Potato Foliage

Berg (1) reports that the tomato strain is less vigorous than the potato strain in its attack on potato foliage, whereas Röder (21) says that the 2 strains are identical in that respect. To compare the virulence of these strains on potato plants, sporangia of each of the potato culture, the natural tomato, and the potato culture passed 10 times through tomato foliage [T(10)], were collected, germinated, and each of the swarm-spore suspensions diluted to approximately the same number of swarmspores. Inoculations were made by placing drops of the suspension on the tips of potato leaves. The lengths of lesions produced were measured 8 days after inoculation. Four plants of the variety Green Mountain were inoculated with each culture.

The incubation period was the same for all cultures. Small lesions were evident on all plants 80 hours after inoculation. As shown in table 2, lesions

TABLE 2.—Comparison of tomato and potato cultures of *Phytophthora infestans* on potato leaves

Culture	Number lesions measured	Length of lesions Mm.
Potato	53	36.68 ± 0.91^a
T(10)	42	36.29 ± 0.76
Natural tomato	47	34.36 ± 1.04

^a Standard error.

produced by all cultures progressed at similar rates. The greatest difference, that of 2.32 ± 1.38 millimeters, between the potato and natural tomato cultures, is not significant, since the D/SE value of 1.68 yields odds of less than 10 to 1. The infected plants were held in the greenhouse 10 days following measurement of the lesions to observe further progress of the disease. The 3 strains of the fungus progressed into petioles and stems and all plants showed similar severity of attack at any given time.

Stability of Tomato Cultures on Potato Foliage and Tubers

At the time of isolation from tomatoes, potato plants were inoculated in the greenhouse with the tomato culture. This culture was carried on potato foliage for slightly over 3 months, during which time it was transferred 12 times. At the 3rd, 6th, 9th, and 12th passages, the fungus was tested on

tomato foliage simultaneously with the same culture that had been grown continuously on tomato foliage. The two cultures attacked tomato foliage with uniform severity, indicating that the tomato culture may exist for long periods on potato foliage with no loss of virulence for tomato.

Immediately upon isolation, the tomato culture was transferred also to potato tubers, where it was grown continuously for 6 months and transferred 22 times. Inoculations of tomato plants, made at approximately monthly intervals, showed that there was no apparent loss of virulence for tomato during the 6-month period of culture on potato tubers.

The potato culture, after 10 passages through tomato foliage, was transferred to potato foliage and to potato tubers. One culture was grown on potato foliage for 64 days (9 transfers), while the other culture was grown on potato tubers for 92 days (12 transfers). Tests of these cultures on tomato foliage showed that, as in the case of the natural tomato culture, there was no less of virulence for tomato foliage by passage through potato foliage or potato tubers.

The results of these experiments in carrying both the natural and the built-up tomato cultures on potato show further the similarity between a natural tomato culture and one built up in the greenhouse, as both are stable for a considerable period of time on potato foliage and tubers. This agrees with the results of Berg (1) and Röder (21), in which Berg maintained a tomato culture on potato tubers for 12 months, and Röder for 24 months, with no loss in virulence for tomato.

MORPHOLOGICAL COMPARISON OF CULTURES

The tomato and potato strains of *Phytophthora infestans* appear identical, morphologically, when grown on potato tubers or leaves. Sporangial measurements (100 sporangia of each strain) show (Table 3) that the potato

TABLE 3.—Lengths of sporangia, in microns, of potato and tomato strains, produced on potato tubers, potato and tomato leaves, at 18° C.

Host	Strain		Difference
	Potato	Tomato	
Potato tuber	29.34 ± 0.34 ^a	29.08 ± 0.52	0.26 ± 0.62
Potato leaves	26.60 ± 0.39 ^c	26.14 ± 0.35	0.46 ± 0.52
Tomato leaves	24.46 ± 0.44 ^c	26.35 ± 0.40	1.89 ± 0.59 ^b

^a Standard error.

^b Significant, with odds greater than 100:1.

^c Difference, 2.14 ± 0.59, significant, with odds greater than 100:1.

and tomato strains produced sporangia of equal size, when grown on potato tubers, or on potato leaves. When grown on tomato leaves, sporangia of the potato strain were significantly smaller than those of the tomato strain. Another comparison shows that, whereas the tomato strain produced sporangia of similar size on both potato and tomato leaves, the potato strain produced significantly smaller sporangia on tomato than on potato leaves.

These comparisons show that the resistance of tomato leaves tends to reduce sporangial size of the potato strain, and that the two strains produce sporangia of equal size when grown on a completely susceptible host.

FIELD OBSERVATIONS

On September 25, 1938, late blight was observed in a field of tomatoes at Ithaca. At that time, the disease affected only scattered plants throughout the field. A careful inspection of diseased plants showed that lesions were present only on large, mature leaves. Although there were many blighted leaflets per plant, the infection was so light that the disease was not noticeable from a distance of a few feet. No lesions were found on stems, apical leaves or fruits. Several diseased leaves were placed in a moist chamber in the laboratory, where fair sporulation occurred. Using sporangia from these leaves, tomato plants were inoculated in the greenhouse, and the infection produced was similar to that from a potato culture passed 2 or 3 times through tomato foliage.

From September 25 until October 29 the field was examined at weekly intervals. It appeared evident that virulence was increasing, because at the end of that period fruit rot was excessive, infection was widespread, and many plants were dead. Diseased fruit were then collected and a culture of *Phytophthora infestans* was isolated that was carried for 6 months on tomato foliage in the greenhouse. There was no apparent increase in virulence from the additional passages through tomato foliage, the culture always producing typical tomato-blight infection.

DISCUSSION

In view of results herein reported, it appears permissible to conclude that the tomato strain originates in nature from successive passages of the potato strain through tomato foliage. The 3 questions asked in the introduction can now be answered. (1) Tomato blight is more serious in the vicinity of blighted potatoes because blighted potatoes must furnish the original inoculum for the tomatoes. (2) Tomato blight occurs later than potato blight because a certain amount of time is required for the build-up of the tomato strain. In greenhouse experiments, the potato culture has been passed through tomato foliage 7 times in 32 days. This agrees well with Berg's statement that in the field tomato blight usually occurs from 4 to 6 weeks later than potato blight. The same reasoning explains why tomato blight was unknown in Australia prior to the discovery of *Phytophthora infestans* on potato; and why tomatoes blighted in Texas in 1931. (3) The fungus overwinters in potato tubers as the potato strain. There is, to be sure, no reason why the tomato strain could not overwinter in potato tubers. It has been proved in this paper, and by Berg and Röder, that the tomato strain retains its virulence for tomato after long existence in potato tubers. However, it is believed that potato tubers are rarely infected with the tomato strain, at least in the North. This is based on the fact that unusually favorable conditions for blight must exist for a long period to permit the build-up of the tomato strain in the field. Under such favorable

conditions, the potato foliage would be dead with blight by the time the build-up was attained, so that there could be no spread of the tomato strain to the potato. It has been observed many times that tomato blight is comparatively rare in the North, and it is the belief of the writer that the tomato strain must be built up anew for practically every epiphytotic of tomato blight in this region. Potato blight appeared unusually early (end of July) in western New York in 1938 and was exceedingly severe throughout the season, yet it was late September before blight became prevalent in tomato fields in this region. Had the tomato strain of the fungus been present in July, it seems certain that tomato blight would have appeared at a much earlier date, since weather conditions were so favorable.

No certain explanation of the reason for the more or less regular appearance of tomato blight in regions like West Virginia and Virginia can be offered at this time. It seems probable that either weather conditions are more favorable than in the North for the build-up of the tomato strain, or that the tomato strain is carried over more regularly in potato tubers. It could be that fall crops of potatoes, in regions where they are grown, become infected from diseased tomatoes, if a blighted summer crop of potatoes were present to furnish inoculum for the tomatoes. Berg (1) suggests that the tomato strain might live over in the soil in milder climates, whereas it could not survive the cold winters of the North.

A type of biologic specialization exists here that is not comparable with the condition that that term has come to connote. It is not permissible, therefore, to refer to the tomato "race" of *Phytophthora infestans*. Reddick (17) points out that *P. infestans* exhibits a low order of parasitism, as shown by its wide host range and outright killing of the host. The indications are that the fungus is sufficiently unstable in its parasitic behavior to be able to adapt itself rather easily to a new set of conditions necessary for the maintenance of its existence.

SUMMARY

Tomato foliage is resistant to the potato strain of *P. infestans*, but susceptible to the tomato strain. Six or 7 consecutive passages of the potato strain through tomato foliage increased the virulence of the fungus so that it readily killed tomato plants. Additional passages through tomato foliage, from the 8th to the 22nd, inclusive, brought no further virulence increase.

A culture, virulent for certain potato hybrids, gave the typical "potato strain" reaction on tomato foliage. After 7 passages through tomato, it had acquired virulence for tomato equivalent to the tomato strain while maintaining its original virulence for the potato hybrids.

Potato and tomato strains attacked potato foliage with equal vigor.

Tomato strains retained their virulence for tomato after growing 3 months on potato foliage, and after 6 months on potato tubers.

Tomato and potato strains produced sporangia of the same size on potato leaves, and on potato tubers, but the potato strain produced smaller sporangia than the tomato strain on tomato leaves.

It is concluded that the tomato strain arises in nature as a result of serial passage of the potato strain through tomato foliage.

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A LABORATORY BIOLOGICAL ASSAY OF TENACITY OF FUNGICIDES

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INTRODUCTION

It is common knowledge that the higher the tenacity² of protective fungicides the longer they will be effective under field conditions. Information is limited, however, regarding the factors involved in tenacity and their evaluation by accelerated biological techniques.

The usual laboratory methods of assaying the tenacity of fungicides are (A) to determine chemically the amount of residue remaining from the initial deposit on a sprayed surface after it has been subjected to a weathering test and (B) to determine biologically the fungicidal value³ of the initial and weathered deposits. The chemical method measures only the quantity of the initial deposit that adheres during weathering, and the biological method measures only the decrease in fungicidal value. There is no method that measures in one test both the quantity and fungicidal value of the deposit adhering during weathering. Such a method is needed.

Essentially all laboratory weathering tests (washing and "artificial rain") may be divided into 3 groups: (A) immersion of the sprayed surfaces in water; (B) immersion of the sprayed surfaces in water and removal with subsequent shaking; (C) atomizing, spraying, or dropping water on to the sprayed surfaces, so-called "artificial rain." All the methods have their advantages and limitations, but none combines the features of simplicity, rapidity, and effectiveness.

The objects of the present investigation were to develop a simple, rapid, and effective laboratory washing test that will give reproducible results and to develop methods for evaluating data on tenacity.

MATERIALS AND METHODS

The fungicide deposits were subjected to the washing test described below to determine their tenacity. This test is a modification of the washing test described in Group B and simulates the washing and beating action of rain in nature. The conditions of the test are reproducible.

A standard moist chamber half (221 mm. × 75 mm.) was filled with water. Two sprayed slides that had been dried for 1½ hr. were placed back to back, the sprayed surfaces facing outward, and were held with one corner between the thumb and first two fingers. They were immersed for $\frac{1}{3}$ of their length at one side of the moist chamber, drawn across rapidly to the other

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² Tenacity is the ability of a fungicide deposit to resist weathering (2).

³ Fungicidal value is the ability of a chemical to kill or inhibit the growth of a fungus spore (3).

side, raised from the water, given a sharp jerk, immersed again, drawn rapidly back across to the opposite side, raised, given a sharp jerk, and immersed again. This process was repeated until 20 strokes, 10 in each direction, had been made.⁴ The time required was 30 seconds. For the sake of brevity, this technique will be called the "rapid test" throughout this paper.

The fungicidal value of the unwashed (initial) and washed deposits was assayed by methods recently described (3).

The spray materials used were those under test for fungicidal value while this work was in progress.

EXPERIMENTATION

Comparison of Weathering Tests

On Plain Glass Slides. Eight slides were sprayed with each of 3 organic fungicides and allowed to dry for $1\frac{1}{2}$ hr. Two of each were then atomized with water for 10 min., 2 were immersed in water for 10 min., 2 were given 20 strokes in the rapid test, and 2 were left untreated. The slides were then dried again for $1\frac{1}{2}$ hr., after which the spore suspension was added. The indicator fungus was *Alternaria solani*, and the spore suspension was adjusted to 5,000 spores per cc. (Table 1).

TABLE 1.—Comparison of 3 tests for removing fungicide deposits on plain glass slides

Material	Percentage spores inhibited after ^a			
	No test	Atomizing	Immersion	Rapid test
Check	9.0	10 min.	10 min.	30 sec.
Organic 1	86.0	42.0	24.0	32.0
Organic 2	92.0	45.0	54.0	30.0
Organic 2 plus Bentonite-lime	90.0	86.0	71.0	79.0

^a Based on 600 spores, 100 counted in each of 6 drops.

The data on spore inhibition indicate that, in general, the tests did not differ radically among themselves as to effectiveness in removing the various deposits from a plain glass surface. The time required differed considerably, however, for only 30 seconds were required in the rapid test. Time is important to the technician.

On Coated Glass Slides. Since slides coated with cellulose nitrate (1) are now frequently used, the effectiveness of the rapid and atomizing tests in removing deposits on that surface was compared. Eight slides were sprayed with each of 2 copper compounds, Apple Coposil and Z-O, to give deposits that would inhibit approximately 100 per cent of the spores. After drying $1\frac{1}{2}$ hr., 2 slides of each were given 20 strokes and 2 of each 40 strokes in the

⁴ It was found that the amount of wash-off increased with increasing number of strokes up to 20 and did not greatly increase with further additional strokes. Thus, 20 strokes lies at the point where the wash-off curve, under the conditions of this test, flattens out.

rapid test, 2 were atomized with water for 60 sec., and 2 left untreated; the coating was unaffected by 20 strokes, loosened somewhat by 40, and loosened considerably when atomized for 60 sec. After the slides had been dried again for 1½ hr. the spore suspension was added. The indicator fungus was *Macrosporium sarcinaeforme* Cav., and the spore suspension was adjusted to 5,000 spores per cc. (Table 2).

TABLE 2.—*Comparison of two tests for removing fungicide deposits*

Material	Test	Spores inhibited ^a
		<i>Per cent</i>
Check	None	7.0
Apple Coposil	None	98.0
	20 strokes	37.0
	40 strokes	28.0
	Atomizing	25.0
Z-O	None	98.0
	20 strokes	27.0
	40 strokes	19.0
	Atomizing	18.0

^a Based on 600 spores, 100 counted in each of 6 drops

The data show that 40 strokes in the rapid test were as effective in removing deposits from the coated surface as was atomizing for 60 seconds; 20 strokes in the rapid test were slightly less effective. It should be mentioned here that 2 slides can be used at once in the rapid test, whereas only one slide at a time can be used in the atomizing test for reproducible results.

Determination of Tenacity

The applicability of the rapid test in determining the tenacity of spray materials was investigated. The experiments were made as follows: A paired series of coated slides was sprayed with the material under test, using a precision sprayer (3), in such a way as to give deposits of known quantity over the range of 0 to 100 per cent spore inhibition. The slides were then dried 1½ hr., after which one slide of each pair was subjected to 20 strokes in the rapid test. The fungicidal value of the unwashed and washed deposits was then determined in the usual way. The indicator fungus was *Macrosporium sarcinaeforme*, and the spore suspension was adjusted to 5,000 spores per cc.

The tenacity of the following compounds was determined: Bordeaux, red copper oxide, yellow copper oxide, Basicop, Compound A, Copper Hydro "40," Coposil CDV, and Z-O. The detailed data for 2 of the materials is presented as an example of the type of data obtained (Table 3). The evaluation of the data will be discussed in the next section.

These data show that the rapid test removed some of the deposit, for the percentage spore inhibition was lower after washing than before.

TABLE 3.—Data on tenacity of two copper compounds, Compound A and Coposil CDV

Material	Cu deposit	Percentage spore inhibition ^a	
		Before washing	After washing
	<i>micrograms/cm²</i>		
Compound A225	23.0
	.345	40.0	13.0
	.450	50.0	16.0
	.680	65.0	35.0
	.900	79.0	45.0
Coposil CDV496	69.0	10.0
	.744	91.0	32.0
	.992	98.0	41.0
	1.488	100.0	55.0
	1.984	100.0	83.0

^a 100 spores counted, 50 in each of 2 drops of spore suspension.

Evaluation of Tenacity Data

An attempt was made to evaluate the tenacity of the materials by means of the decrease in spore inhibition after washing. It was soon found in comparing two materials, however, that equal reduction in spore inhibition after washing indicated equal reduction in tenacity only when the spore inhibition on the unwashed deposits was equal. This is true because the relation of spore inhibition (mortality) to dosage (deposit) is not linear.

McCallan and Wilcoxon (5) devised a method of determining and evaluating data on the tenacity of spray materials. By keeping the spraying time constant, 12 seconds, and varying the concentration in the spray tank, they determined the concentration necessary to give LD50⁵ on sprayed slides before and after 1 minute of "artificial rain." The data were presented in a bar diagram, using the LD50 value as the standard for comparison.

This method could not be used for evaluating the data on tenacity obtained in the experiments reported herein, for in these experiments the concentration in the spray tank was held constant and the spraying time varied. Thus, the fungicidal value was based on the deposit of toxicant (Cu) on the sprayed slide, not on the concentration of toxicant in the spray tank.

The tenacity of the materials was evaluated in the following manner: The data on spore inhibition for both the unwashed and washed deposits were plotted on the same sheet of logarithmic probability paper,⁶ using the ordinate for percentage spore inhibition and the abscissa for original deposits in both cases. A straight line was fitted by inspection to each set of data for the points between 10 and 90 per cent spore inhibition. The line for the washed deposits is below that for the unwashed deposits, showing that the washing test removed some of the original deposit. The LD50 point on each line was determined by interpolation or extrapolation. These two

⁵ LD50 means "lethal dose 50 per cent."

⁶ The use of this paper for plotting spore inhibition data has recently been suggested by Wilcoxon and McCallan (6).

points provide the information for calculating the tenacity. The LD50 deposit for the unwashed slides was divided by the LD50 deposit for the washed slides. The quotient, a decimal figure, measures the amount of the deposit adhering during washing. A quotient of 1.00 (unity) signifies that none of the deposit is removed during washing, whereas a quotient of .40 means that 40 per cent of the deposit adhered. Thus, the quotient is a measure of the relative tenacity and it is termed the "tenacity coefficient." As all the points on the straight lines have equal weight, any point can be used with equal accuracy for comparative purposes. Whatever point is used, however, the tenacity coefficient will be the same, provided the slopes do not differ significantly.

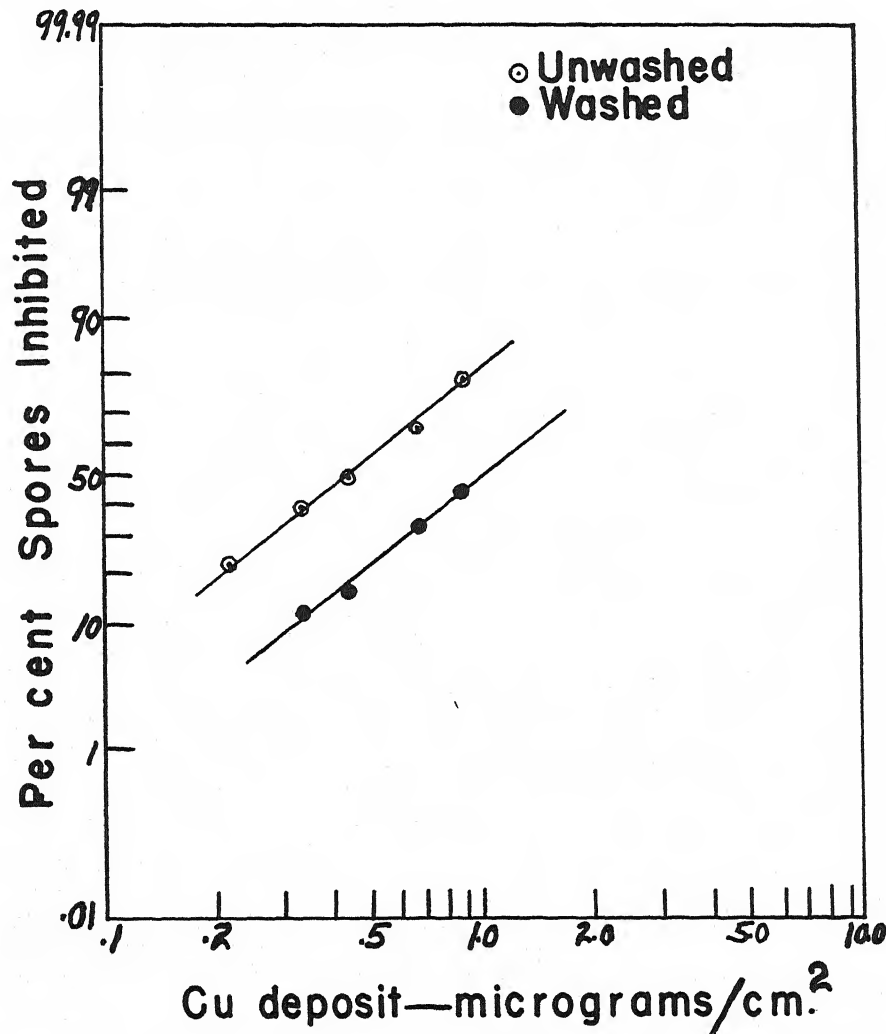


FIG. 1. Fungicidal value curves for unwashed and washed deposits of a copper fungicide plotted on logarithmic probability paper.

The data obtained on a copper compound in a typical experiment are shown plotted on logarithmic probability paper in figure 1. The tenacity coefficients of the copper materials tested are listed in table 4. The reproducibility of data is shown by the fact that in experiments on 3 different days the tenacity coefficient was .466, .450, and .486 for Compound A and .867, .844, and .853 for red copper oxide.

The Bordeaux coefficient⁷ also may be useful in calculating the tenacity coefficient. In using it to calculate the tenacity coefficient, the Bordeaux coefficient of the unwashed and washed deposits may be obtained independently, keeping as the point of reference in both cases the unwashed deposit of a standard fungicide (3). The tenacity coefficient is obtained by dividing the Bordeaux coefficient for the washed deposit by that for the unwashed deposit. This procedure will frequently save the labor of making the tests on washed and unwashed deposits the same day.

TABLE 4.—*Tenacity coefficients of copper compounds, 1939 samples*

Material	Tenacity coefficient
Bordeaux880 ^a
Red copper oxide855 ^a
Yellow copper oxide834 ^b
Basicop544 ^a
Compound A467 ^a
Copper Hydro "40"340 ^a
Coposil CDV333 ^a
Z-O316

^a Average of 3 tests.

^b Average of 2 tests.

The order of tenacity of Bordeaux, red copper oxide, and Coposil CDV is the same as that reported by Magie and Horsfall (4), based on chemical analysis of deposits before and after rain, for similar materials in 1934 and 1935 on apple and cherry foliage in the field. With the exception of Basicop, the order of tenacity of the materials is essentially the same as that reported by McCallan and Wilcoxon (5), based on chemical analysis of deposits on sprayed slides before and after 1 minute of "artificial rain," for similar materials in the laboratory in 1937. This agreement is rather striking, in view of the well-known variations in proprietary materials.

The copper compounds tested did not contain large amounts of spreader. The drops of spore suspension spread to the same diameter on both the unwashed and washed deposits for all the materials. When materials containing large amounts of spreader are used, a correction in the data is necessary as the drops of the spore suspension do not cover the same area on the unwashed and washed deposits because the washing test removes much of the spreader. Thus, the same number of spores are exposed to different amounts of deposit.

⁷ The Bordeaux coefficient is the ratio of the spore-inhibiting power of a fungicide under test to that of a standard fungicide (3). It is independent of the day-to-day variations in the indicator fungus and the date of testing.

DISCUSSION

The main features of the washing test for fungicide deposits described herein are simplicity, rapidity, and effectiveness. Comparisons with other tests have shown its practicability. The conditions of the test are reproducible. It is ideally suited for use with coated slides having films that cannot stand long exposure to water. No correlation has been made, as yet, between the number of washing strokes and the number of inches of rainfall in the field; but, on the other hand, neither has a correlation between "artificial rain" and natural rainfall been established.

A biological method of assaying the tenacity of fungicide deposits measures the quality of these deposits, *i.e.*, the spore-inhibiting power, whereas a chemical method gives a quantitative measure of the deposits, *i.e.*, the amounts before and after weathering. The method of determining and evaluating the tenacity of fungicide deposits described herein permits a measure of both the amount and the quality of these deposits before and after washing. The measure of the quality is provided by the spore inhibiting powers of the unwashed and washed deposits; the measure of the quantity is provided because the amounts of the unwashed deposits are known and the amounts of the washed deposits can be determined from their spore-inhibiting powers. In this method, however, the assumption is made that washing did not fractionate or otherwise change the deposit in any way—that the deposit after washing contains the same chemical in the same form, *etc.*, as before washing. No method to date, however, provides any information on this point.

The tenacity coefficient gives a numerical rating to the relative tenacity of a material in the same way that Bordeaux coefficient gives a numerical rating to the fungicidal value. Its reproducibility indicates that it, too, is independent of day to day variations in the indicator fungus and the date of testing. By the use of these two coefficients, it may be possible to predict the protective value of fungicides in the field under varying climatic conditions. The value of such predictions, if they can be accurately made, is obvious.

The method of assaying the tenacity of fungicides described herein may serve as a useful research tool in studies on the phenomena involved in the weathering of a fungicide deposit. It is, by its nature, most useful in measuring the direct effects of washing, but it will also serve, however, to distinguish between the effects of other factors involved.

SUMMARY

A simple, rapid washing test for fungicide deposits has been developed. Comparative tests show that it is as effective as other tests in removing fungicide deposits.

Details are given for a laboratory biological assay of the relative tenacity of fungicides based on the spore-inhibiting powers of deposits of toxicant before and after washing. This method makes possible a numerical rating

for tenacity. The term tenacity coefficient is applied to this numerical rating.

The relative tenacity of several copper compounds has been determined by this method. The order of tenacity of these materials closely approximates that reported for similar materials, based on chemical analysis of deposits before and after weathering, in the laboratory and field.

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COTTON SEED DUSTING IN RELATION TO CONTROL OF SEEDLING INFECTION BY RHIZOCTONIA IN THE SOIL

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Numerous tests have shown that ethyl mercury phosphate and ethyl mercury chloride effectively control cotton seedling infections by seed-borne fungi. Marked improvements in stand are observed when anthracnose-infested cotton seed is dusted with preparations containing these materials. In the field, however, many of the seedlings die before or after emergence as the result of attack by fungi present in the soil. It has been suggested that the increase in stand of cotton seedlings noted in certain field tests may have resulted in part at least from control of *Rhizoctonia* and other soil-borne fungi by the organic mercurials applied to the seed before planting (2). To what extent are these dusts effective as fungicides when applied to seed? The experiments here described were made in hope of finding an answer to this question in reference to the fungus *Rhizoctonia solani*, a commonly observed parasite of cotton seedlings.

METHODS AND MATERIALS

The experiments were made in the greenhouse. Equal parts of a grey sandy-loam soil and river sand were thoroughly mixed, steamed for 1 hour,

and placed in clean wooden flats that had been washed with formaldehyde solution. The soil in half the flats was inoculated by stirring into each flat 2 sand-wheat-bran cultures (about 200 cc. by volume) of *Rhizoctonia solani*. The culture of the fungus used had originally been obtained as an isolate from a diseased cotton seedling.

The seed used in these experiments was approximately 2 years old. By reason of its age it was at the time of making the tests almost free of the anthracnose fungus, but carried some *Fusarium moniliforme*, *Fusarium* spp., *Aspergillus* spp. and *Bacterium malvacearum*. The seed was thoroughly mixed to obtain a uniform sample and divided into 2 portions. One portion was left nontreated; the other was dusted with New Improved Ceresan at the rate of 3 oz. per bushel in tests 1 and 1a and 1½ oz. in 2 and 2a. The dusted and nondusted lots of seed were planted in alternate rows lengthwise cross 2 flats placed end to end. Fifty seeds were planted in each row and 8 replications were made on the inoculated and on the uninoculated soils. Thus there were 4 seed and soil combinations in the tests, i.e., nondusted seed planted in noninoculated soil; dusted seed planted in noninoculated soil; nondusted seed planted in inoculated soil; and dusted seed planted in inoculated soil.

The greenhouse was equipped with automatic steam-heat control. For experiments 1 and 1a, planted on March 1 and March 23, respectively, an effort was made by manipulation of ventilators to hold temperatures of the house at 70–75° F., but during the midportion of sunny days the temperature rose to 80 or 85° F. Mid-day temperatures for tests 2 and 2a, planted June 30 and August 7, usually mounted to 95 or 100° F., and night temperatures were also considerably higher than those for experiments 1 and 1a.

All seed apparently having germinated, counts were made of the total number of seedlings emerged, the number emerged and still living, and the number free of stem lesions. The results obtained were analyzed by the analysis-of-variance method. The data are summarized in tables 1, 2, and 3.

RESULTS

Table 1 shows the data obtained for total emergence. Here all seedlings that emerged are included regardless of whether or not any died after emergence. In experiment 1, planted March 1, a significant difference was obtained between treated and untreated seed in both noninoculated and inoculated soil. The difference in the inoculated soil containing *Rhizoctonia* was roughly 5 times as great as in the noninoculated. The calculated F value for interaction between seed treatment and soil inoculation is 47.9, a value much greater than 8.02 required for high significance.

On March 23, a day after removal of the seedlings from experiment 1, this soil was replanted without renewed inoculation. The results are recorded as experiment 1a. There was obtained again a significant difference between total emergence of nondusted and dusted seed on both the noninoculated and inoculated soils. The difference was not relatively greater, however, on one

TABLE 1.—*Effect of a 5 per cent ethyl mercury phosphate dust applied to cotton seed for control of Rhizoctonia infection as indicated by percentage seed emergence*

Sublot No.	Soil and seed treatment	Seedlings emerged (percentage of seed planted)			
		Exp. 1	Exp. 1a	Exp. 2	Exp. 2a
1	Soil not inoculated, seed not dusted	83.3	77.3	76.3	85.5
2	Soil not inoculated, seed dusted	94.2	88.0	92.8	87.3
3	Soil inoculated, seed not dusted	36.5	74.0	20.8	81.5
4	Soil inoculated, seed dusted	84.8	84.5	77.8	88.3
Diff. req. for signif., odds 99:1		10.8	10.7	8.2	7.8
Cal. F value for soil inoculation ^a		109.0	3.1	293.1	0.1
Cal. F value for seed treatment ^a		121.0	31.8	318.6	0.7
Cal. F value for soil × seed ^a		47.9	0.2	96.8	2.4

^a Value required for significance: 4.32 at odds 19:1, or 8.02 at odds 99:1.

than on the other soil in this experiment. The F value for variance between treated and nontreated seed is highly significant, but the corresponding F values for soil inoculation and for interaction between soil inoculation and seed treatment are not significant statistically. Thus contrary to the results of experiment 1, no control of *Rhizoctonia* by the dust used on the seed was indicated by experiment 1a.

For experiment 2, the soil used in experiment 1 was resteamed and reinoculated as in preparation for experiment 1. The seed was planted on June 30. During the period of this experiment the prevailing greenhouse temperatures were considerably higher than for experiments 1 and 1a. As shown in table 1, a significant difference between emergence of treated and nontreated seed was obtained on both soils, and again, as in experiment 1, the increase from seed treatment was relatively much greater on the soil containing *Rhizoctonia* than on the noninoculated soil. The calculated F value, 96.8, high compared to the required 8.02 for significance of interaction between soil inoculation and seed treatment, indicates some retardation of seed rotting by the dust on the treated seed.

Forty days after inoculating the soil for experiment 2 (13 days after removal of the seedlings of experiment 2) the soil was replanted without reinoculation for experiment 2a. In this experiment, no highly significant difference was obtained between dusted and nondusted seed on either soil, and, as in experiment 1a, only an insignificant amount of interaction is indicated between soil and seed treatment.

Many of the seedlings included in the total emergence counts of table 1 died after they had pushed their cotyledons above the soil. The dead seedlings were subtracted from the total emergence counts and the data for the remainders, representing surviving seedlings, are recorded in table 2. In both experiments 1 and 2, in which the seed was planted soon after inoculating the soil, the percentage of living seedlings was strikingly lower, as was to be expected, on the inoculated soil (lots 3 and 4) than on the noninoculated (lots 1 and 2), but there was still a statistically, highly significant difference between treated and nontreated seed on both soils. The calculated F values

TABLE 2.—Effect of a 5 per cent ethyl mercury phosphate dust applied to cotton seed for control of *Rhizoctonia* infection as indicated by seedling survival

Sublot No.	Soil and seed treatment	Seedlings surviving (percentage of seed planted)			
		Exp. 1	Exp. 1a	Exp. 2	Exp. 2a
1	Soil not inoculated, seed not dusted	82.0	77.3	77.0	83.8
2	Soil not inoculated, seed dusted	94.3	87.8	92.0	87.3
3	Soil inoculated, seed not dusted	6.2	52.3	0.8	63.8
4	Soil inoculated, seed dusted	26.7	67.8	18.0	77.5
Dif. req. for signif., odds 99 to 1		9.7	14.0	6.5	8.7
Cal. F value for soil inoculation ^a		877.0	41.3	2155.0	15.8
Cal. F value for seed treatment ^a		45.9	13.8	99.2	47.2
Cal. F value for soil × seed ^a		2.8	0.5	0.5	5.7

^a Value required for significance: 4.32 at odds 19: 1, or 8.02 at odds 99: 1.

for interaction between soil inoculation and seed treatment, however, are below the values required for significance. In experiments 1a and 2a, planted 40 or more days after inoculating the soil, highly significant differences between treated and nontreated seed occurred only on inoculated soil. The F values for interaction between seed treatment and soil inoculation, however, indicate no significance in experiment 1a and only questionable significance in experiment 2a. These results are taken to indicate that, so far as number of living seedlings as distinct from total seedlings emerged is concerned, the fungicide applied to the seed was not significantly more effective in one soil than in the other and failed to give much if any protection against damping-off of seedlings by *Rhizoctonia*.

A considerable proportion of the seedlings counted and recorded as living in table 2 had actually been attacked by *Rhizoctonia* in the inoculated soil and bore sore-shin lesions on their stems at the soil level. Under field conditions some of these would have died, others would have lived to produce stunted, weak or late fruiting plants, others may have shown little harmful

TABLE 3.—Effect of 5 per cent ethyl mercury phosphate dust applied to cotton seed for control of *Rhizoctonia* infection as indicated by disease-free seedlings

Lot No.	Soil and seed treatments	Seedlings disease-free (percentage of seed planted)			
		Exp. 1	Exp. 1a	Exp. 2	Exp. 2a
1	Soil not inoculated, seed not dusted	82.0	65.7 ^b	73.5	83.3
2	Soil not inoculated, seed dusted	94.3	81.7 ^b	91.0	87.3
3	Soil inoculated, seed not dusted	0.5	22.7	0.0	37.5
4	Soil inoculated, seed dusted	4.3	35.7	0.0	49.3
Dif. req. for signif., odds 99 to 1		6.6	12.2	6.4	11.4
Cal. F value for soil treatment ^a		2678.0	219.5	2560.0	216.0
Cal. F value for seed treatment ^a		23.3	23.3	28.9	7.6
Cal. F value for soil × seed ^a		6.6	0.2	28.9	1.8

^a Value required for significance: 4.32 at odds 19: 1, or 8.02 at odds 99: 1.

^b The noninoculated flat became contaminated at one corner by *Rhizoctonia*, and sore-shin lesions occurred on some of the seedlings, hence the lower percentage of disease-free seedlings here than in table 2.

effect. Chief dependence for a profitable yield in the field, however, must be placed on the healthy or disease-free seedlings. Table 3 gives the number of disease-free seedlings remaining after discarding all seedlings that died or developed sore-shin lesions after emergence. For the noninoculated soil these figures differ but little from those in table 2 representing living seedlings. On the inoculated soil, however, the number of disease-free seedlings is nearly or quite equal to zero in experiments 1 and 2, and approximately half the number of living seedlings on the less highly infested soil of experiments 1a and 2a. The calculated F factors indicate significant variance of disease-free seedlings between the 2 soils and between dusted and nondusted seed in all four experiments. For interaction between soil inoculation and seed treatment, however, the F factors fail to indicate significantly better performance of dusted than of nondusted seed in relation to soil inoculation.

DISCUSSION

The F values calculated and recorded in table 1 for significance of interaction between soil inoculation and seed treatment indicate high significance in experiments 1 and 2, planted soon after inoculating the soil. On the other hand, only insignificant differences are indicated for experiments 1a and 2a, planted 40 or more days after inoculating the soil. The emergence of the undusted seed in the inoculated soil increased from 36.5 per cent and 20.8 per cent in experiments 1 and 2, respectively, to 74 per cent and 81.5 per cent in experiments 1a and 2a. This indicates a marked quantitative reduction of the parasite present in the time elapsing between the first and the second plantings in the same soil. A number of workers have presented evidence to show quantitative reductions in *Rhizoctonia* as a result of antagonistic or competitive action between *Rhizoctonia* and other soil microorganisms. Weindling (4) showed that *Rhizoctonia* may be parasitized and destroyed by *Trichoderma lignorum* and suggested the possibility of the control of parasitic soil organisms by inoculation of soil with this fungus. Allen (1) and Haenseler found that seed rotting and damping-off of cucumber and pea seedlings due to *Rhizoctonia* and *Pythium* were reduced by inoculating the soil with *Trichoderma*. Sanford and Broadfoot (3) found that a number of fungi and bacteria were able to reduce pathogenicity of *Ophiobolus graminis*. Porter (2) found that certain bacteria, mixed with *Helminthosporium* or *Fusarium* inhibited or reduced pathogenicity of these fungi for their susceptible plants. In the experiments herein reported the reason for the greater number of healthy seedlings in the second plantings as compared to the first plantings is not clear. The steamed soil was not purposely inoculated with organisms that might be antagonistic to *Rhizoctonia*. Some spore-forming bacteria destructive to *Rhizoctonia* may have survived the steaming of the soil, or fungi may have been added in the tap water used to water the plants or on the seed planted in the steamed soil. Whatever may have been the cause of this reduction in fungus concentration or virulence between the first and second plantings of seed in these tests, it appears

from the data presented in table 1 that ethyl mercury phosphate dust on the planted seed did condition a relatively greater improvement in seedling emergence as distinguished from seedling survival in freshly inoculated than in noninoculated soil. This may be explained on the assumption that the mercury vapors prevented attack and destruction of the seedling radicle tips immediately upon extrusion from the seed coat and thus gave the seedlings a growing start which enabled them to emerge before being overtaken by the fungus. This advantage for emergence was not shown, however, when the same soils were replanted without reinoculation after the first crop of seedlings had been removed (Exp. 1a, 2a). Apparently, the quantity of *Rhizoctonia* in the soil had decreased between the first and second plantings, and, presumably, the seed was in less intimate contact with mycelium of the fungus at the time the radicles pushed out of the seed coats. The advantage of superior emergence shown by the treated seed in the freshly inoculated soil was soon lost and did not follow through to give significantly better improvement in percentage of living or of disease-free seedlings in the freshly inoculated than in the noninoculated soils. In the freshly inoculated soil the seedlings were attacked by *Rhizoctonia* when the radicles had grown beyond the range of fungicidal concentration of the mercury vapors. While in all experiments the treated seed planted in inoculated soil produced a greater percentage of living seedlings than nontreated seed in the same soil and a greater percentage of disease-free seedlings in the plantings made in the less heavily infested soil, these increases appear to be more intimately related to effect of the dust on the seed itself, or on fungi infesting the seed, than to protection against fungi in the soil. It was not found that the increases in living or disease-free seedlings resulting from seed treatment were significantly greater in inoculated than in noninoculated soil. Thus it appears from the results of the tests described herein that the organic mercury dust used on the seed in these tests may be, so far as final stands are concerned, of little, if any, value in control of infections from *Rhizoctonia* in the soil.

SUMMARY

Nondusted cotton seed and that dusted with a preparation containing 5 per cent ethyl mercury phosphate, were planted in steamed soil that had been inoculated with cultures of *Rhizoctonia solani*. Controls of nondusted and dusted seed were run in noninoculated soil. Counts to determine total emergence, living seedlings, and disease-free seedlings were made.

The dusted seed showed significantly greater improvement in seedling emergence on inoculated than on noninoculated soil when the seeds were planted soon after adding the fungus to the soil, but not when several weeks had elapsed between the time of soil inoculation and seed planting.

The number of seedlings that lived after emergence, also the number that escaped stem infections, were increased by seed treatment in all experiments but the increase was not relatively greater by a statistically significant amount on inoculated than on noninoculated soil.

The results of the tests indicate that, so far as final stands of seedlings are concerned, organic mercury dust applied to cotton seed before planting may be of little, if any, value as a protectant against *Rhizoctonia* in the soil. Its protective action against fungi carried on the seed is not questioned.

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RELATIONS OF PEDICULOPSIS GRAMINUM AND FUSARIUM POAE TO CENTRAL BUD ROT OF CARNATIONS¹

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Heald (4, 5) first called attention to a bud rot of cultivated carnations caused by a fungus, tentatively identified as a species of *Fusarium*, found in constant association with the mite *Pediculopsis graminum* (Reut.).² In 1908, Heald (6) stated that the *Fusarium* was merely a fortuitous contamination, and that the real pathogen responsible for the rot is *Sporotrichum anthophilum* Pk. Stewart (17), however, showed that *Sporotrichum anthophilum* is a synonym of the earlier described *Sporotrichum poae* Pk., and, owing to the fact that carnation buds may suffer rot from divers agents, proposed for this disease the distinctive name of "Sporotrichum bud-rot." Following Stewart's proposal subsequent investigators have adopted this name for the complex ailment. As Wollenweber and Reinking (21) have recently shown that *Sporotrichum poae* is actually a species of *Fusarium*, the name "Sporotrichum bud-rot" is no longer apt.

Benham and Kesten (1) have shown that several species of *Sporotrichum*, s. str., as well as *Penicillium brevi-compactum*, when artificially introduced into the carnation bud, can produce a disease strikingly similar to "Sporotrichum bud-rot." Wollenweber and Reinking (21, 22) have noted also that *Fusarium reticulatum* from wheat, *F. avenaceum* from apple, and *F. tricinctum* from both apple and *Helianthus* also may cause a similar rot when artificially introduced into carnation buds.

Since a bud-rot of carnations which is due to a true *Sporotrichum* or another species of *Fusarium* may some day be found, and as it may be that *Pediculopsis* likewise distributes the fungus *Acremoniella occulta* Cavares

¹ A portion of the work reported here was done at the University of Rochester, 1938-9.

² Described by Wolcott (20) as *Pediculoides dianthophilus*, but shown by Hodgkiss (17) to be synonymous with *Pediculopsis graminum* (Reut.).

(13), it is difficult to replace the name "Sporotrichum bud-rot" with a more precisely descriptive one. Therefore the carnation bud disease characterized by *Fusarium poae* and its vector *Pediculopsis graminum* may be called central bud rot of carnations. This name is suitable as it implies that the central floral organs of the bud, and not the calyx, are involved in the disease.

DESCRIPTION OF STEWART'S BUD ROT

Young carnation buds infected by *Fusarium poae* and the mite *Pediculopsis graminum* may appear outwardly normal, but when opened show a moist, brownish, decayed mass of the inner floral organs. Pistil, stamens, styles and petal bases may be rotted through by the *Fusarium* which is generally visible as a heavy or sparse, white, cottony growth. Embedded in this decay and mold are found the glistening white, ellipsoid bodies of the pregnant female mites. The pregnant *Pediculopsis* may range from less than 1 mm. in length to, at times, more than 3 mm. in length. Young, severely infected buds generally do not open. Mid-size (about 16–20 mm. long) and large buds may open following even heavy infection by the *Fusarium*. Such buds, when dissected, show also a central decay, a cottony growth of the fungus, attached bodies of pregnant mites, and an accumulation of droplets of water in the rotten mass. Unlike the diseased young buds, large buds show external symptoms of the disease. Not only are they extremely soft or mushy at their bases, but such buds generally fail to unfold at one side as they expand, so that the opened flower has a peculiar lopsided appearance. It is a fact of common record that the white varieties of carnation are most susceptible to this disease, while crimson and dark-red varieties are least affected. Most detailed descriptions of the symptoms of bud rot have been given by Heald (6), and Stewart (17); reliable descriptions and figures of *Pediculopsis graminum* will be found in Reuter's account (15), and in that of Hodgkiss (17); Wollenweber and Reinking (21) describe and figure *Fusarium poae*. Prophylactic and combative measures are described by Heald (6), Stewart (17), Molz and Morgenthaler (10, 11), and Wollenweber and Reinking (21).

DISTRIBUTION OF CENTRAL BUD ROT

Stewart's bud rot of carnations has been reported from greenhouses in Nebraska (4, 5, 6, 20), New York (17), Illinois (3), and New Jersey (18, 19) in this country, in Germany by Molz and Morgenthaler (10, 11), by Reitter (14), and in New South Wales (23). Reitter's case is of extraordinary interest, for it is the only record of the disease on carnations growing in open fields.

Although it is difficult to obtain reliable information regarding the prevalence of the disease from greenhouse owners, it seems that it has not been uncommon sporadically over the past 15 years on Long Island, near Rochester, New York, and in central New Jersey, the only localities in which I have had opportunity to visit houses. In each of these localities a silver top of

common grasses, in which both *Fusarium poae* and *Pediculopsis graminum* were concerned, was not uncommon.

INOCULATION EXPERIMENTS WITH *FUSARIUM POAE* DERIVED FROM GRASSES³

Although all recent authors agree that the carnation (central) bud rot probably originates from diseased grasses, it is hardly certain, for only Stewart's (17) meagre data are available on the pathogenicity to carnations of *Fusarium poae* derived from grasses. It is, therefore, of considerable importance that experiments on a larger scale be pursued. To this end the following experiment was undertaken. The cultures of *Fusarium poae* employed had been isolated from various common grasses (*Poa*, *Phleum*, *Agrostis*, etc.) suffering from silver-top. Unfortunately, varietal names for the carnations used in this and the following experiments were not available.

Seventy-nine buds located on 20 plants of a yellow carnation flecked with pink were selected for the experiment, measured in silhouette, and labelled. Fifty buds, distributed among the 20 plants, were lanced and slightly lacerated with a sterile needle. A small tuft of *Fusarium poae* was introduced into each wound. Eighteen control buds, located on 16 plants, were similarly lanced and lacerated, but not inoculated. Eleven control buds, each on a separate plant, were not inoculated. The experiment was begun on October 8 and concluded on October 23; during the interval the room temperature was maintained at 12° to 16° C. and the carnations were sprinkled at least every other day. The first clear cases of bud rot appeared on October 16.

At the close of the experiment the buds were again measured and their condition recorded. Each of the 79 buds was dissected and separately tested for the presence of *Fusarium poae* by introduction of the bud remnants into sterile slants of potato-dextrose agar. All of the control buds, both incised and nontreated, were completely negative so far as *F. poae* was concerned. Two of the lanced control buds gave cultures of an unidentified mold, but had not shown any symptoms of rot on dissection. Of the group inoculated with *F. poae*, 40, or 80 per cent, were positive; only 1 bud of the 40 failed to show visible evidences of bud rot when dissected. The 10 negative buds failed to give *F. poae*, despite the fact that 3 slants were taken for each bud.

Two late buds of a pink variety growing on a neighboring bench were taken with bud rot. Dissection of the buds revealed a growth of mold that, when cultured, proved to be *F. poae*. Thrips present in these buds were the only possible disseminating agents that could be discovered. It may be of interest that a case of *Anaphothrips* associated with *F. poae* has been reported (21, 22).

The measurements of the buds gave interesting data. Firstly, the size of the bud at the time of the inoculation apparently but little affects the course of the disease. Each size class of bud contributed to the positive

³ It is my pleasure to thank Dr. O. A. Reinking of the New York Agricultural Experiment Station, Geneva, New York, for kindly verifying the identity of the cultures of *Fusarium poae* (Pk.) Wr. used in these experiments.

group a percentage very little different from the percentage it represented of the entire group originally inoculated. Secondly, only the smallest buds were retarded externally in their development. The measurements showed that the sheaths of buds inoculated at a length of 12 mm. or more, in spite of an internal rot, appear to grow equally with those of the control series (compare 7). Thirdly, buds of sizes greater than 20×10.5 mm. almost invariably tended to open by the close of the experiment, in spite of pronounced central rot.

These data amply confirm Stewart's (17) observation, based on only 5 more or less successfully inoculated carnation buds, that *Fusarium poae* recovered from grasses is truly pathogenic to carnation buds, and produces a bud rot not different from that of *F. poae* originating from diseased carnation buds. As no other agent was involved in this bud rot, *F. poae* by itself—once within the tissue of the bud—produces the entire range of symptoms of the disease.

“Weakened” Strains of *Fusarium poae*

One strain of *Fusarium poae* had been maintained in the laboratory for more than 3 years (2) on Difco potato-dextrose agar without repassage through normal hosts, and no longer showed vigorous growth on this medium. Other strains also were more than a year removed from their original isolation and, likewise, grew poorly. Accompanying the loss of vigor appeared to be a decreased capacity of the *Fusarium* to color the surface of the medium an intense violet-red. It was believed that inoculation of carnations with these strains would be of especial interest, for reculturing the fungus from diseased carnation buds should demonstrate any possible revivifying effect on a strain of its passage through a plant host.

The effect on the formerly “weakened” strains of *Fusarium poae* of a short residence and growth in the carnation bud was striking but not entirely consistent. More than half of the recovered *F. poae* returned to vigorous, cottony growth and colored the potato-dextrose medium intensely. The effect, however, was not lasting, and the strains soon declined to their former irregular and retarded growth characteristics.

THE RELATIONSHIP OF THE MITE PEDICULOPSIS GRAMINUM TO CENTRAL BUD ROT

Although generally reliable sources of reference (9, 12, 16, 21) are quite definite in ascribing the transmission and introduction of the bud-rot fungus to the mite *Pediculopsis graminum*, as well as attributing a symbiotic relationship between the mite and the fungus, the published data do not justify such finality of statement.

Wolcott (6) held that *Pediculopsis graminum* is the sole agent causing the bud rot of carnations, but Heald (4, 5) and Stewart (17) very quickly showed that a fungus is the true pathogen. Nevertheless, as Heald (4, 5), Stewart and Hodgkiss (17), and Davis (3) pointed out, the rot always starts from the center of the buds and does not grow externally on the buds under natural conditions. As the mite was invariably found to accompany the

fungus of bud rot, and as Stewart had also found *Pediculopsis graminum* frequently accompanying the *Fusarium* on grasses suffering from silver top, it was quite naturally concluded that *Pediculopsis* is the active agent in introducing the *Fusarium* spores into the heart of the bud. Experiments to test this inference (6, 17, 23) nevertheless failed.

Molz and Morgenthaler (10) found that apparently spontaneous outbreaks of *Fusarium poae* on their supposedly sterile media in Petri dishes were brought about by *Pediculopsis graminum* creeping into them. Furthermore, they state that they found bits of mycelium and spores of *F. poae* stuck to the bodies of the mites. This evidence, they hold, shows the mite to be capable of distributing the fungus. In addition they consider the relation to be a symbiotic one, for the fungus in turn is said to afford the mite nutriment by decaying the bud tissues or the media. Nevertheless, it remains to be proved that the mite actually is instrumental in bringing about infection of the carnation buds with *Fusarium*. To this end the following experiment was performed.

Four varieties of carnations (white, white flecked with pink, yellow flecked with pink, and dark crimson) growing on 2 benches were employed. After carefully cleaning the benches and checking the plants for complete absence of bud rot and *Pediculopsis*, hundreds of actively wandering mites from 6 tubes of *Pediculopsis graminum* cultured on *Fusarium poae* (growing on potato-dextrose agar) were released on the soil of each bench on October 23. Two days later 9 buds were chosen at random and dissected. Two of these (22 × 12 mm. and 26 × 13 mm.) already had mites in their hearts. By October 29, 4 of 19 buds examined possessed mites and fungus; 1 of these had a luxuriant growth of *Fusarium* and female mites undergoing physogastric. On this day 6 more cultures were liberated on each bench. On December 3, 22 buds of the flecked yellow variety were examined; of these only 3 had advanced stages of bud rot. Twenty-two buds of the white variety gave only 3 cases of bud rot; 28 buds of the flecked white carnation disclosed 12 cases of bud rot; and only 2 of 28 buds of the dark crimson variety were afflicted with bud rot. In all, 119 buds were examined and 24, or 20 per cent, of these had bud rot. Both *Fusarium* and *Pediculopsis* were recovered from each of the diseased buds on slants of potato-dextrose agar; in no case was the *Fusarium* alone recovered.

It may be added that an experiment in progress when the beds were cleaned gave, so far as could be ascertained, results similar to the above. In addition it should be mentioned that, regardless of dense cotton plugs, mites are able to creep into and infect sterile media with *Fusarium poae*, as Molz and Morgenthaler maintain.

From these data it can scarcely be doubted that *Pediculopsis graminum* is the vector for *Fusarium poae* as causal agent of central bud rot of carnations.

It is clear that *Fusarium poae* must be introduced into the tissues themselves, for mere stuffing of fungus into the healthy bud without laceration

of the tissue does not result in bud rot. Apparently the gnawing of the tender tissues by the female nymphs of *Pediculopsis* produces the wounds necessary for successful inoculation of the *Fusarium*. Undoubtedly the failure of one series of Hodgkiss' (17) experiments is attributable to the fact that he introduced into the buds chiefly immobile pregnant females that no longer needed to feed. As the young of these females were released in the bud from their mother's bodies, they were, quite obviously, free from all natural contacts with *Fusarium*. In spite of any activities of the young mites, no bud rot developed. Hodgkiss also performed a series of inoculations with "a goodly number of crawling females and pure cultures of the fungus." Here again no typical cases of bud rot were initiated. Very likely the crawling females used were from isolated mothers; as the mites had no previously established contacts with the *Fusarium*, and the *Fusarium* had no source of nutriment, no success of the experiment was to be expected.

Since they found no injury of the *Fusarium* by the mite, Molz and Morgenthaler (10) believed that *Pediculopsis graminum* feeds only on the decay or nutriment released from the substrate by *F. poae* in their plate cultures. However, their figure 1 of a Petri culture of the fungus infected with mites belies their belief. As may readily be ascertained from such cultures, mites frequently attach to the fungus where the *Fusarium* has appressed against the upper glass cover. Although the fungus is nourished by the medium below, no decomposed soups of the latter reach the mite here. Nevertheless, the mite becomes bloated with food materials. It seems clear, then, that in this case it receives its nourishment directly from the substance of the *Fusarium* growing about it.

As physogastric or swelling females are always found attached to portions of buds heavily invaded by *Fusarium poae*, and as they are never found on nearby fungus-free tissues, it seems unlikely that the pregnant females of *Pediculopsis graminum* feed on healthy tissues. Additional support that the mite in advancing pregnancy does not gain its nourishment from normal tissues follows from the fact that Hodgkiss does not report finding any physogastric female descendants in his negative buds, in spite of the abundance of healthy tissues present.

One further criticism of Molz and Morgenthaler's opinions must be made. Korff (8) and many recent authors have reported *Weissährigkeit* accompanied by attacks of *Pediculopsis graminum* common in German meadows and grainfields. Therefore, it seems doubtful that, as Molz and Morgenthaler (10) have concluded, the appearance of central bud rot in Germany has resulted from importation of carnations from North America.

SUMMARY

Reasons are advanced for renaming the "*Sporotrichum* bud-rot" of carnations central bud rot.

Fusarium poae isolated from grasses produces the typical "*Sporotrichum* bud-rot" of carnations, as Stewart indicated.

Initial size of the carnation bud at the time of infection with *Fusarium poae* little affects the course of the disease, and, in spite of an internal rot of the bud, growth of the calyx seems unretarded for at least 16 days following infection.

It is proven that the mite *Pediculopsis graminum* can and does introduce *Fusarium poae* into carnation buds, producing central bud rot.

A type of antagonistic symbiosis exists between *Pediculopsis graminum* and *Fusarium poae*, for it is clear that the mite can subsist on the fungus alone.

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SOIL FUMIGATION WITH CHLOROPICRIN AND CARBON BISULPHIDE TO CONTROL TOMATO ROOT KNOT AND WILT¹

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(Accepted for publication April 2, 1940)

Chloropicrin (CCl_3NO_2) and carbon bisulphide (CS_2) were tested in soil-fumigation experiments for the control of tomato (*Lycopersicon esculentum* Mill.) root knot caused by *Heterodera marioni* (Cornu) Goodey, and tomato wilt caused by *Fusarium lycopersici* Sacc. From similar experiments, Howard, Stark, and Smith (3) concluded that complete eradication of nematodes was not necessarily the most profitable in tomato production, for dosages of chloropicrin that delayed initial nematode infection until an extensive fibrous root system had developed, were sufficient to produce normal yields. Watson and Goff (4) described soil treatment with sodium cyanide, and expressed the opinion that nematodes can spread through the soil at a rate of about a foot per month by their own movements. Godfrey (1) controlled nut grass with chloropicrin and cited earlier work on soil fumigation. Preliminary reports on soil fumigation were given by the writer (5, 7).

MATERIALS AND METHODS

The materials and methods were similar to those described by Godfrey and Young (2). In practical use, dosages of chloropicrin and carbon bisulphide should depend on the spacing of the injection holes and the nature and temperature of the soil. Consequently, the following formula was derived to convert figures of pounds of fumigant per acre into cubic centimeters of fumigant per hole: $\frac{abc}{d} = e$, where a is the area of the plot, b is the number of cubic centimeters of fumigant per pound, c is the number of pounds of fumigant per acre, d is the number of holes, and e is the number of cubic centimeters of fumigant per hole. For the experiments described in tables 1 to 3, this formula was applied to the injection of chloropicrin into plots 2.5×20 ft. with holes 15 inches apart:

$$\frac{0.001148 \text{ A.} \times 274.9 \text{ cc. of } \text{CCl}_3\text{NO}_2 \text{ per lb. at } 20^\circ \text{ C.} \times 100 \text{ lb. per A.}}{32 \text{ holes}} =$$

about 1 cc. of chloropicrin per hole. Substituting 359.2 cc. of carbon bisulphide per pound at 20° C. in this formula, gives about 13 cc. of CS_2 per hole at 1000 lb. per acre.

The plots were prepared in a field of Norfolk fine sandy loam, which was abundantly infested with both root-knot nematodes and *Fusarium lycopersici*. The treated plots had borders of boards 4 to 10 in. (usually 8 in.)

¹ Published with the approval of the Director of the Texas Agricultural Experiment Station as Contribution No. 595, Technical Series. The chloropicrin and some other materials used in the experiments described here were donated by Innis, Speiden & Co., of New York City. The CS_2 and some other materials were donated by the Freeport Sulphur Co. of New York City.

deep and projecting 2 in. above the soil. Treated plots and nontreated check plots were distributed at random in the field. When nearly dry, the soil was loosened to a depth of 8 in. and treated with the disinfectant. The temperature of the soil ranged from 65° to 95° F. when treated. Immediately after treatment, the soil in certain plots was covered with glue-coated paper for 4 to 10 days to delay the escape of the fumigant. In other plots, the top half-inch of soil was wetted, the fumigant was injected, and then the soil was soaked with water to a depth of 2 to 3 inches (both chloropicrin and carbon bisulphide being practically insoluble in water).

In Series 1 to 3, the soil plots were covered as designated with glue-coated kraft paper. Glue-coated tar paper was used in Series 4. In Series 5 to 8, saxolin plain duplex paper with animal glue (Chase Bag Co., Goshen, Indiana) was used. Comparative plots were covered with duplex paper having the sheets held together with vegetable paste, casein, or asphalt (Arkell Safety Bag Co., New York City). For the plots 2.5 ft. wide, paper covers 4 ft. wide were used, with the edges buried deeply in the soil. Paper 5 ft. wide was used on the plots 4 ft. wide in Series 7.

Injection holes were 15 in. apart, beginning 3 to 7 inches from the margins of the plots, except in comparative plots, where the injection holes were 12 in. apart. No consistent differences in nematode control were noted between these 2 spacings. Fumigants were injected 8 in. deep in the soil, and the holes were closed by stepping on them immediately. A tube-peg board was constructed for injecting chemicals into the soil in 1936. In 1937 and 1938, fumigants were injected with a Carbona prod. For accurate measurement with this instrument, it was necessary to use a mixture of chloropicrin and gasoline (1:3). A Vermorel Pal injector and an Isco Larvjector were used in 1939.

For comparison with the chloropicrin and carbon bisulphide treatments, other plots were treated with formaldehyde, sodium cyanide, cyanamid, and sodium hydroxide. Formaldehyde (40 per cent) was injected at the rate of 1000 lb. per A. into holes in 3 plots and mixed with the soil in 2 other plots. For disinfection with hydrocyanic acid, the soil of 8 plots was watered with 3 gal. of sodium cyanide solution per plot (applying NaCN at rates of 800 lb. and 1200 lb. per A.) followed by ammonium sulphate (at rates of 1200 lb. and 1800 lb. per A.) after which the plots were watered abundantly. Sodium hydroxide (2 per cent solution) was sprinkled on the soil in 2 plots at a rate of 1470 lb. per A. Powdered Aero Cyanamid was mixed with the surface soil in 2 plots at rates of 1000 lb. and 1500 lb. per A., respectively.

Watermelon seed (var. Dixie Queen) was planted in the plots to determine the effectiveness of the soil treatments in controlling root knot. In addition, Whippoorwill cowpea seed was planted in Series 5; in Series 7, Long Green okra seed was planted. Data from these very susceptible host plants were calculated together in summarizing the experiments. The date of treatment and time of planting and harvesting in each series of experiments are as follows:

Series No.	Date of treatment	Days after treatment		Series No.	Date of treatment	Days after treatment	
		Seed planted	Roots dug			Seed planted	Roots dug
1	8/ 6/36	18	91	5 ^b	3/14/38	31	110
2	9/ 9/36	7	56	6	8/22/38	7	65
3	4/19/37	8	86	7	3/20/39	12	98
4 ^a	7/26/37	11	92	8 ^c	3/22/39	12	100

^a Twenty-five healthy Greater Baltimore tomato seedlings were transplanted into each plot on August 31; final notes on tomato wilt were taken 72 days later.

^b Twenty-five healthy Stone tomato seedlings were transplanted into each plot on April 15; final notes on tomato wilt were taken 75 days later.

^c Twenty-five healthy Earliana tomato seedlings were transplanted into 7 treated plots and 5 check plots on April 3; final notes on tomato wilt were taken 88 days later.

In order to determine the effectiveness of soil fumigation in controlling *Fusarium lycopersici*, very susceptible varieties of tomatoes were transplanted into certain plots (see footnotes a, b, c). When tomato plants developed wilt symptoms, they were recorded and removed from the plots. All weeds were counted and removed from the plots. The tools used in cultivating the plots were disinfected in 5 per cent formaldehyde. Series 2, 4, and 6 were terminated by frosts. Most of the test plants had developed mature fruits by the time final records were taken. The roots of test plants were excavated with a shovel and washed with water to facilitate examination for root knots. The percentages of plants with severe root knot, as shown in the tables, show the economic damage from this disease and the efficiency of the control methods. Root knot was classified as severe when the roots showed many knots, or knots about 1/4 to 1 in. in diameter. Root knot was classified as mild when a plant showed only one or a few knots about 1/32 to 1/8 in. in diameter.

RESULTS

Soil fumigation with 300 to 600 lb. of chloropicrin per acre, in plots covered with glue-coated paper, delayed the appearance of *Fusarium* wilt symptoms in tomatoes from 20 to 40 days and decreased the number of wilted plants to 7 to 10 per cent as compared with 72 to 100 per cent in the nontreated plots (Table 3). Carbon bisulphide showed little effect in controlling tomato wilt. Papers covered with animal glue, casein, or vegetable paste were the most satisfactory of the covers tested in holding adequate concentrations of chloropicrin and carbon bisulphide in the soil (Tables 1, 2). No consistent differences in the thoroughness of disinfection of field plots was found to be attributable to differences in glue, casein, or vegetable paste on cover paper, so the data were calculated together from the groups of plots covered with these materials. When the glue-coated paper was removed from the plots 5 days after treatment, soil treated with chloropicrin retained the odor of the gas. Soil treated with carbon bisulphide, however, had a characteristic odor, unlike this chemical. It was demonstrated that careful watering of the surface soil before and after injection

of the fumigants confined the fumigants satisfactorily in many cases. Paper covered with asphalt was less effective in many cases than glue-coated paper or water.

TABLE 1.—*Effect of soil fumigation under glue-coated paper on root knot*

Fumigant	Lb. per A.	Series	Number of		Percentage of plants infected		Range in percentage of healthy plants
			Plots	Plants	Severe	Mild	
CCl ₃ NO ₂	100, 150	2, 3	2	131	20	18	48 to 74
“	150, 200	5 ^a	5	822	70	22	1 to 84
“	200, 250	3, 4, 7, 8	11	1845	4	12	50 to 100
“	300	2, 3, 6, 7, 8	21	3224	3	10	52 to 100
“	300, 450	5 ^a	12	1570	20	32	9 to 100
“	350 to 750	All but Ser. 5	22	3862	1	3	69 to 100
CS ₂	500	3	3	175	43	35	1 to 57
“	800	5 ^a	3	343	63	34	1 to 6
“	1000	5 ^a	9	1318	13	34	20 to 85
“	1000 to 2250	1, 3, 4, 6	15	1203	0	2	96 to 100
“	3000	5 ^a	3	387	0	8	88 to 98
Checks	0	All	98	6738	63	27	0 to 85

^a Root knot was poorly controlled in several plots in Series 5, probably because of a heavy rain and the long duration of the experiment.

TABLE 2.—*Effectiveness of different soil covers on the control of root knot by soil fumigation*

Chemical	Lb. per A.	Kind of cover	Series	Number of		Percentage of plants infected		Range in percentage of healthy plants
				Plots	Plants	Severe	Mild	
CCl ₃ NO ₂	350	Glue-paper	4	3	169	0	5	93 to 100
		Water	4	4	276	7	15	58 to 87
“	300	Glue-paper	5	9	1135	20	25	9 to 100
		Asphalt-paper	5	3	445	27	38	33 to 69
		None	5	2	146	59	27	9 to 18
“	450	Glue-paper	7	2	771	0	3	84 to 100
		Asphalt-paper	7	1	422	0	13	72 to 99
“	600	Glue-paper	8	2	282	0	4	92 to 99
		Water	8	2	228	1	13	77 to 99
“	350 to 750	Glue-paper	All but Ser. 5	22	3862	1	3	69 to 100
	300 to 600	Water	4, 6, 7, 8	19	3238	1	6	58 to 100
CS ₂	1000 to 2250	Glue-paper	1, 3, 4, 6	15	1203	0	2	96 to 100
	750 to 2000	Water	3, 4, 6	14	1130	0	3	89 to 100
Checks ...	0	None	All 8	98	6738	63	27	0 to 85

Formaldehyde, cyanamid, and sodium hydroxide were ineffective in controlling root knot. Only a few watermelon plants emerged in the plots treated with the cyanamid. The sodium cyanide treatment controlled nema-

TABLE 3.—*Effectiveness of chloropicrin and carbon bisulphide for controlling tomato wilt*

Chemical	Lb. per A.	Series No.	Number of		Plants wilted
			Plots	Plants	
					<i>Per cent</i>
CCl ₃ NO ₂	150, 200	5	6	150	32
“	300 ^a	5	3	59	24
“	250 to 600	4, 5, 8	22	450	9
CS ₂	750 to 3000	4, 5	26	613	82
Checks	0	4, 5, 8	35	469	95

^a Plots covered with asphalt-coated paper; all other treated plots were covered with glue-coated paper.

todes in some cases, but the soil of treated plots was unusually hard and showed some salty patches, and watermelons grew poorly in the treated plots.

Chloropicrin and carbon bisulphide were used also in experiments to control damping-off of tomato seedlings, as mentioned in a preliminary report (6). The soil had an inoculum potential of nearly 100 per cent due to the presence of species of *Pythium* and *Rhizoctonia*. It was fumigated with 10 cc. of chloropicrin per cubic foot, or with 1 cc. of carbon bisulphide per pound of soil. Chloropicrin fumigation controlled damping-off and facilitated the production of good tomato seedlings when the light intensity was adequate. Fumigation with carbon bisulphide was ineffective in controlling damping-off fungi.

DISCUSSION

Since variation is likely to occur in field plots, the last column in the tables was prepared to give the range in percentages of plants free from root knot in the different plots. Root knot was perfectly controlled in many of the fumigated plots. Methods of completely controlling soil-inhabiting parasites in fields usually are too expensive to be profitable, so methods of soil fumigation are planned to secure practical control of parasites at moderate cost. The experiments here described were conducted in an open field where it was possible to take only a few of the precautions against reinfection of the soil by nematodes. The nematodes may travel naturally through many inches of warm, moist, sandy soil within 3 months, and such distribution may be aided by growth of the roots of the host plant through the soil. Pocket gophers carry soil and travel extensively underground, and may pass through plots without disturbing the soil surface. It is likely that nematodes or the wilt fungus may have moved into some of the treated plots from the surrounding untreated soil within 100 days after disinfection, despite the wooden borders, disinfection of tools, and other precautions. Without using very costly and strict methods, one may not expect uniformly perfect control of these parasites by disinfection of the soil in open fields.

Chloropicrin, injected into the soil at rates of 300 to 600 lb. per acre, usually controlled most of the weeds present, especially Johnson grass

(*Sorghum halepense* (L.) Pers.), crab grass (*Digitaria sanguinalis* (L.) Scop.), and thorny amaranth (*Amaranthus spinosus* L.). As an exception, weed seeds were poorly controlled in some plots where the soil was too dry when treated. Besides the above tests, chloropicrin treatment (400–600 lb. per A., covered with glue-coated paper) gave satisfactory practical results in 5 hotbeds, 10 coldframes, and one greenhouse bed.

SUMMARY

Heterodera marioni, *Fusarium lycopersici*, and weeds usually were controlled in soil fumigated with chloropicrin at rates of 300 to 600 lb. per acre.

Root knot usually was controlled in soil fumigated with carbon bisulphide at rates of 1000 to 3000 lb. per acre. However, this chemical controlled neither tomato wilt nor weeds.

As a coating for paper used in covering treated soil, animal glue, casein, and vegetable paste were all adequate and equally effective in delaying the escape of chloropicrin and carbon bisulphide. Wetting the top 2 or 3 inches of soil with water was almost as effective as covering the soil with glue-coated paper.

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OCCURRENCE OF BIG BUD OF TOMATO IN THE PACIFIC NORTHWEST¹

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A tomato disease, with symptoms characteristic of big bud (4), appears to be very sparingly distributed in the Pacific Northwest. This disease was first observed in September, 1937, reappeared in September, 1938, and was again observed in July, 1939. In the 4 locations where it was observed a trace to one per cent of the plants were affected. Losses were negligible. Marglobe, Dwarf Champion, Bonny Best, and miscellaneous lots of *Lycopersicon* spp., collected by H. L. Blood in South America, 1937-1938, were affected. A similar disease (1), producing witches'-brooms and phyllody, appeared at the same time on common bean (Fig. 3), Lima bean, soy bean, alfalfa, sweet clover, carrot, and squash.

SYMPTOMS

Symptoms of big bud, as the disease appeared on tomato in this section, have rather closely paralleled those described by Samuel, Bald, and Eardley (4) for big bud on tomato in Australia. In every case infection appeared late in the growing season. Effects were most pronounced on late growth at the top of the plant or on new shoots. A greatly enlarged calyx with segments united was found on earlier flower clusters or on the oldest flower of later clusters (Fig. 1). Carpels in these phylloid flowers with greatly enlarged calyses were not usually phylloid. In younger flowers calyx segments were separated and petals and carpels were more phylloid.

Diseased Marglobe plants in one location bore abnormal fruits, which appeared to be prematurely and unevenly ripened. Placental tissue was more or less woody (2, 3), and flavor, as well as texture, was impaired.

Phloem proliferation (Fig. 2) was constantly associated with the abnormal inflorescences and witches' brooms. This is perhaps the most important diagnostic feature for identification of the disease. This adventitious phloem tissue was largely composed of the companion type of cells. The virus seemed to stimulate development of adventitious buds into shoots; however, these shoots failed to continue vigorous growth, possibly because of the difficulty of food movement in the abnormal stem tissues.

BIG BUD DISTINCT FROM CURLY TOP

The new disease as first observed in the field in 1937 and again in 1938 was intermixed with curly top. In 1939, however, separate occurrence of

¹ Data presented are based on investigations carried on by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating with the Oregon Agricultural Experiment Station and the Umatilla Field Station. Published with the approval of the Director of the Oregon Agricultural Experiment Station as Technical Paper No. 335 from the Division of Botany.

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FIG. 1. Big bud on tomato. Note enlarged calyx on oldest flower of the inflorescence and phyllod sepal and petals of the second flower.

big bud was noted in fields where curly top was not found. Big bud on tomato appears to stimulate and enhance vegetative growth, while the curly-top virus causes tissue necrosis and depresses vegetative activity. On tomato the two diseases may usually be differentiated on the basis of gross symptoms. Diagnosis is more certain if histological comparisons of phloem development are made.

TRANSMISSION

Successful transmission of the disease on tomato to tomato was accomplished by setting buds from diseased shoots into stems and petioles of healthy plants. A month elapsed after inoculation before symptoms were detected. Out of 24 plants inoculated, 5 developed phyllody and foliage symptoms and 15 others produced foliage symptoms of big bud. Twenty-three noninoculated plants remained healthy.

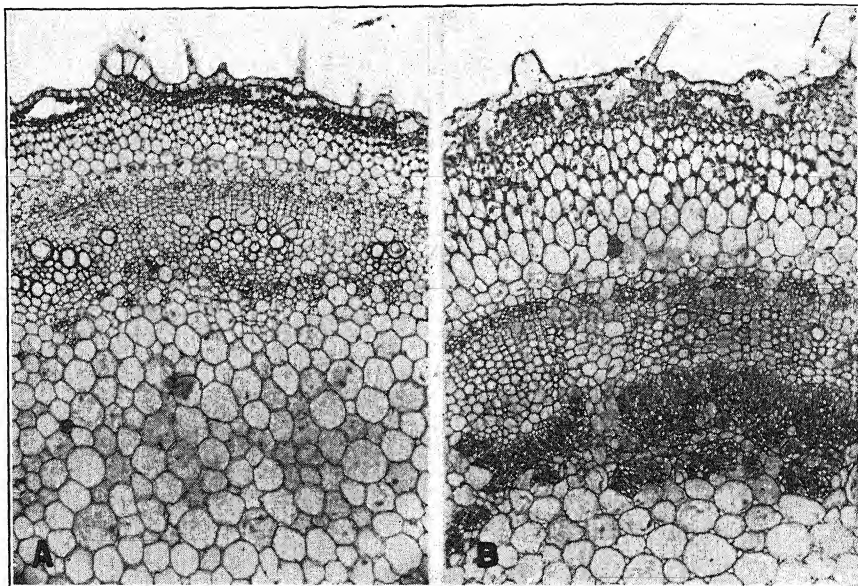


FIG. 2. A. Cross section of normal tomato stem. B. Cross section showing phloem proliferation characteristic of big bud on tomato.

Juice transfers were attempted from tomato to tomato, common bean, Lima bean, soy bean, carrot, and night shade, *Solanum villosum*. Carborundum was employed in this technique. Results in each case were negative.

PHYLLODY ON OTHER HOSTS

Phyllody similar to that produced on tomato by big bud has occurred

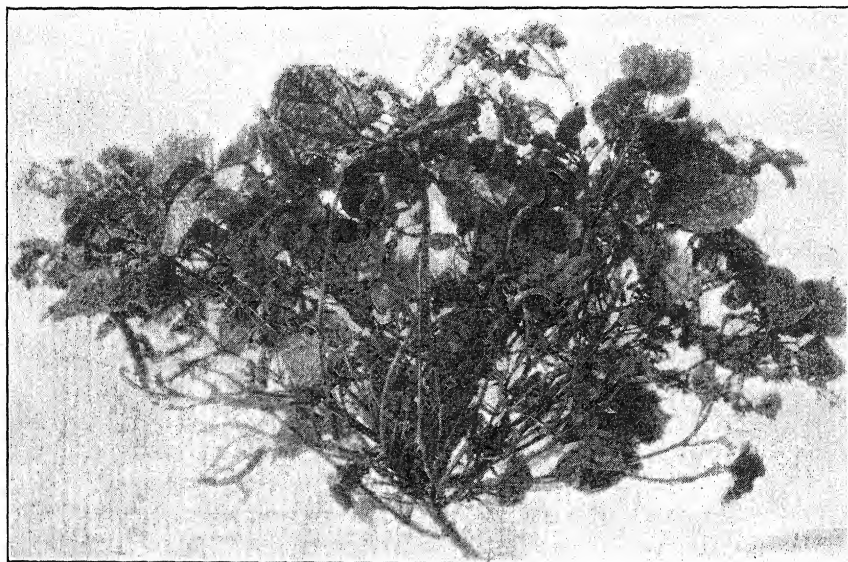


FIG. 3. Phyllody on common bean.

on common bean (Fig. 3), Lima bean, soy bean, alfalfa, sweet clover, carrot, and squash. Phyllody on these crops also appeared late in the growing season and was associated with late branching, renewed growth, and witches' brooms. Diseased plants were examined for starch accumulation in pith cells. Such starch accumulation was described by Samuel *et al.* (4) and Michailova (2) as characteristic of big bud on tomato and woodiness on bindweed. Heavy starch accumulation was found in diseased common bean and a little starch in soy bean, but none was discovered in the other crops. Phloem proliferation did not accompany phyllody on the various crops. However, there seemed to be some increase of xylem. This is characteristic of woodiness (big bud) on bindweed (2). These comparisons suggest a relationship between big bud and phyllody on common bean and possibly also for phyllody on the other crops.

SUMMARY

Big bud of tomato occurred very sparingly in the Pacific Northwest during late summer and fall of 1937, 1938, and 1939. Marglobe, Dwarf Champion, Bonny Best, and strains of *Lycopersicon* spp. were affected and exhibited symptoms similar to those previously described for big bud. Phloem proliferation is a characteristic of this disease and with other symptoms serves to distinguish big bud from curly top. Buds from diseased plants transferred the disease to healthy tomato plants. A disease similar to big bud also occurred on other hosts.

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THE PRODUCTION OF APOTHECIA OF SCLEROTINIA SCLEROTIUM AND S. TRIFOLIUM IN CULTURE¹

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Numerous methods have been used for the production of the perfect stage of various organisms from sclerotia, but no technique has been developed that

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

does not require attention at frequent intervals. Recognizing that the essential feature was the maintenance of a constant moisture supply over a long period of time, a method was developed whereby this could be done under sterile or semi-sterile conditions. It is the object of this paper to describe the method and some results obtained with it.

REVIEW OF LITERATURE

Wadham (18) reported the production of apothecia of *Sclerotinia trifoliorum* if the sclerotia were planted on wet cotton wool in sterile culture tubes. Godfrey (8) and Ramsey (16) noted the production of stipes and apothecia by *S. ricini* and stipes or "horn-like outgrowths" by *S. sclerotiorum* in old cultures on potato-dextrose agar. Burger (2, 3) obtained the production of stipes of *S. sclerotiorum* on cracked corn and sterile soil substrata. Burgwirtz and Eremeyeva (4) observed that the sclerotia of *S. sclerotiorum* produced stipes unless a very rich nutrient medium was used, but Soursac (17) obtained no germination of the sclerotia of this fungus in various media. Kheswalla (13) and Mundkur (14) obtained stipes of *S. sclerotiorum* on potato-dextrose and Kotila's agars, and potato-dextrose and corn-meal agars, respectively.

Many investigators have used moist sand or soil as a substratum for the production of apothecia of *Sclerotinia sclerotiorum* and *S. trifoliorum*. Moist sand or soil also has been used as a substrate for the production of apothecia of the brown-rot fungus (7, 10, 15), *S. convoluta* (6), *S. gladioli* (5), *S. minor* (1, 12), and the perfect form of *Botrytis cinerea* (9).

TECHNIQUE

In the present studies a satisfactory technique for the production of stipes and apothecia of *Sclerotinia sclerotiorum* and *S. trifoliorum* has been developed (11). The technique consists essentially in placing sclerotia on a medium that will maintain them at the proper moisture content for apothecial development, without further attention, for several months. The medium used is sterile 1 per cent agar in water, slanted in 2 × 8.5 cm. vials tightly plugged with cotton. Mature sclerotia from pure cultures are pressed into the agar aseptically, the vials plugged tightly, and placed under any desired conditions of temperature and light, where they may be kept without further attention for 6 months or longer. Some have produced apothecia after being outdoors for a year or more.

Sclerotia of both species produced stipes and matured apothecia in vials when placed outdoors at Lexington, Kentucky (Fig. 1). *Sclerotinia trifoliorum* started stipes and matured apothecia most abundantly during October, November, and December; whereas *S. sclerotiorum*, planted on agar at the same time in late summer, generally developed stipes and apothecia in February, March, and April. Thus a fundamental difference in these two species in the time required to initiate stipes was discovered by this technique.

Sclerotia from 105 isolates of *Sclerotinia trifoliorum* and 28 of *S. sclero-*

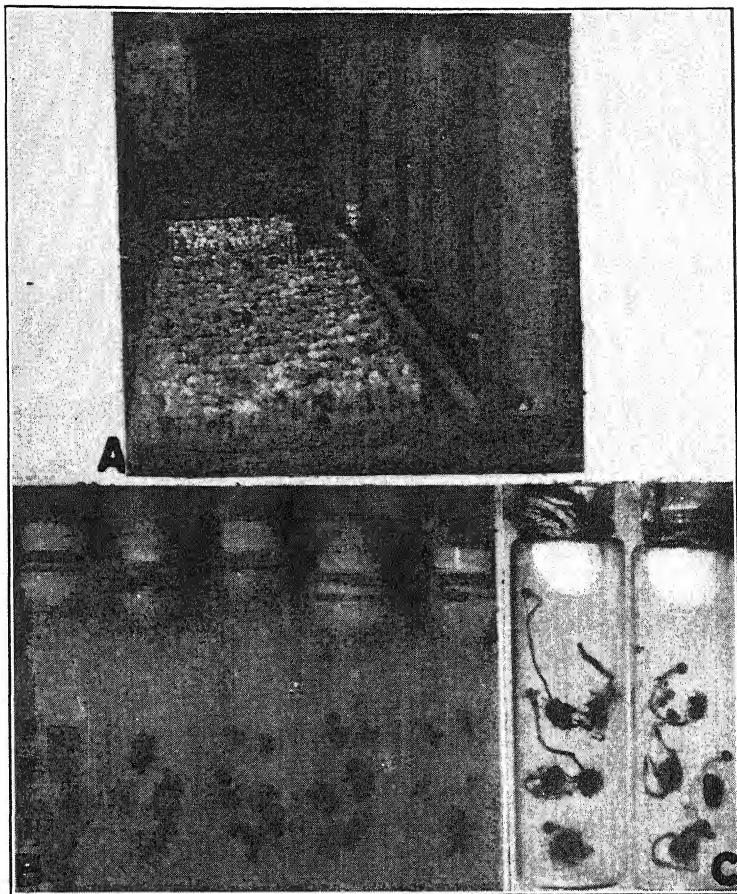


FIG. 1. A. Cellophane covering (right) and vials with *Sclerotinia sclerotiorum* and *S. trifoliorum* outdoors at Lexington, Ky. B and C. Stipes and apothecia of *S. trifoliorum* and *S. sclerotiorum*, respectively, on 1 per cent water agar.

tiorum, in vials that were placed outdoors at intervals between August 31, 1934, and October 11, 1935, were studied for stipe and apothecial development. Three to 5 sclerotia of each isolation were planted from 1 to 35 different times. Germination generally was obtained from 75 to 85 per cent of the cultures planted at any one time. Sclerotia from some cultures, however, have never produced stipes, and a few that produced them in the earlier tests failed in the later studies. The reason for this failure is not known. Each sclerotium of the isolations that germinated matured an average of more than 1.5 apothecia.

Stipes of *Sclerotinia trifoliorum* were initiated in incubators in which temperatures of 4°, 10°, 14°, and 18° C. were maintained, but *S. sclerotiorum* produced stipes only at 10° and 14° C. Both species started stipes in an ice refrigerator in which the temperature varied between 9° and 11° C. Fourteen degrees C. appears to be about the optimum temperature for the

production of stipes by these *Sclerotinias*. *S. trifoliorum* required an average exposure, at 14° C., of about 15 to 20 days, and *S. sclerotiorum* 45-50 days to produce stipes. The time required varied, however, depending somewhat upon the previous treatment of the sclerotia. At 14° C. the shortest exposure after which they produced stipes was 6 and 21 days, respectively. This is in agreement with the field observations, *i.e.*, that *S. trifoliorum* produces its apothecia in the fall and *S. sclerotiorum* remains dormant through the fall and produces apothecia in the spring, in Kentucky.

Only one abnormal apothecium was obtained from thousands of stipes produced and kept in darkness; however, when placed in diffuse sunlight they matured normal apothecia unless temperature was a limiting factor. Thus these studies emphasize the importance of moisture, temperature, and light in the production of apothecia of these *Sclerotinias*.

This technique has also been used for the production of the perfect stage of *Claviceps purpurea*. Sclerotia from rye kept dry over summer were planted on water agar in vials September 11 and some placed outdoors and some in a cold room at 3° C. About the middle of April, a part of both those in the cold room and those outdoors had produced stipes, while those outdoors had also matured and discharged ascospores. Some of these had germinated on the walls of the vials. Some of the sclerotia were overgrown by other fungi and failed to produce stipes. This technique should prove of value in the study of any organism in which the perfect stage is expected to arise from a sclerotium. With modifications in the material used for the vial the method could be used successfully in a study of the effects of various light rays or quality of light on apothecial development.

The water-agar technique has several advantages over the commonly used sand or soil method. They are as follows: 1. Uniform moisture is maintained for a relatively long time without attention. 2. Sclerotia may be watched, without disturbing them, for the first appearance of stipes. 3. Large numbers of sclerotia may be studied in a comparatively limited space and with very little attention. 4. The effects of time, temperature, and light may be studied independently. 5. Contaminating organisms do not grow well, and mycelial growth of *Sclerotinias*, if any, is usually sparse. 6. This method affords an opportunity for a study of the cytology and genetics of these organisms.

SUMMARY

The method most commonly used for production of the perfect stages of *Sclerotinias* consists of planting sclerotia in sterile sand or soil kept moist by frequent watering, but other substrata have been used. A more satisfactory technique which consists in planting mature sclerotia in 1 per cent water agar in tightly plugged vials is described and its advantages noted. It also has been used successfully for the production of the perfect stage of *Claviceps purpurea* and is suggested as a method for study of other sclerotial forms.

With constant moisture, temperature and time of exposure are important factors in the initiation of stipes of *Sclerotinia sclerotiorum* and *S. trifoliorum*. The optimum temperature for the production of stipes by these species is about 14° C. A much longer exposure at this temperature is necessary for initiation of stipes of *S. sclerotiorum* than of *S. trifoliorum*. Light is not necessary for growth of stipes but is apparently necessary for the production of apothecia. Apothecia mature normally in the vials.

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PROPAGATION OF SOUR CHERRIES BY PIECE-ROOT GRAFTING TO AVOID SPRAYING SEEDLING STOCKS FOR LEAF SPOT

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(Accepted for publication April 10, 1940)

Cherry varieties are propagated commercially by budding on Mazzard (*Prunus avium* L.) or Mahaleb (*P. mahaleb* L.) seedlings. Considerable information has been accumulated on the relative merits of these rootstocks (1, 2, 8, 9, 11, 12, 14). In choosing between them, the decision is influenced

sometimes by regional requirements for the orchard trees, but more often by the practical limitations and conditions incident to propagation in commercial nurseries. The failure to control the leaf-spot disease (*Coccomyces hiemalis* Higgins) on Mazzard seedlings lined out for budding is frequently the limiting factor in obtaining satisfactory stands.

Although experimental and other evidence generally favors the use of Mazzard seedlings, there is no common agreement as to the superiority of one stock over the other in orchard performance (12) and, in the absence of a specific demand, most nurserymen prefer using Mahaleb stock. Because this stock is more readily "worked" and also withstands the climatic conditions of the North Central States, particularly, it is commonly used for propagating cherries in the United States (14). Mazzard stock is used to a considerable extent, however, in plantings of the coastal regions of the Pacific Coast and in Eastern States, where climatic variations are not so extreme. It usually produces a larger and longer-lived tree in the orchard.

From the nurseryman's point of view, the well-known susceptibility of Mazzard seedlings to leaf spot makes the use of this stock unprofitable. Under average conditions numerous spray applications are necessary to obtain even partial control and generally sufficient defoliation occurs to make budding operations partial or even complete failures.

In an attempt to eliminate the undesirable and at the same time retain the desirable qualities of Mazzard rootstocks, it was reasoned that propagation by piece-root grafting might be resorted to. Obviously, propagation by this method would (1) eliminate the necessity of spraying seedlings lined out for budding, (2) avoid the hazards in budding operations due to weather conditions, (3) protect the relatively "tender" seedling part of the tree (4, 5, 6) by placing it approximately 6 inches below the ground level and (4) afford an opportunity for the development of scion roots, which are generally considered relatively hardy (6, 10, 15).

That propagation by grafting was practiced to a limited extent before 1900, is indicated by Craig (6); but, by 1903, according to Price and Little (13), this method was "... little practiced. ..." No other reports on propagation by piece-root grafting have been noted in a limited survey of the literature, but other workers have reported on experiments involving collar grafts made on lined-out seedlings (7) and on dormant stem grafting of Mazzard seedlings (3).

EXPERIMENTS

Preliminary experiments in grafting included sweet and sour cherry varieties on Mazzard and Mahaleb seedlings. Both whip-and-tongue and wedge grafts were made with piece-root cuttings taken from the proximal (collar) region and from cuts taken approximately 3 inches below the collar. The results of several years' experience indicated that better stands were secured with the sour cherry varieties than with sweet varieties and with grafts made from root pieces taken from the collar region, i.e., the "top cut" of the seedling roots as compared with root pieces taken farther down the

axis. Better stands also resulted with the use of Mazzard seedlings in comparison with Mahaleb seedlings.

The procedure in the experiments reported here consisted in (a) collecting scion wood in the late fall from 2-year nursery trees, (b) making the grafts relatively early in the season (February 1) and (c) storing the grafts at a sufficiently low temperature (approximately 45° F.) to prevent bud pushing.

When the grafts were planted, April 10, 1939, the amount of callus at the union and the degree of "knitting" was rated as slight, compared with the condition generally observed on apple grafts.

The results of this test with sour cherry grafted on Mazzard root pieces are shown in table 1.

TABLE 1.—*Results of piece-root grafting experiments made with Early Richmond and Montmorency cherries, whip-and-tongue grafted on "collar" piece-roots and on piece-roots taken approximately 3 inches below the collar of 1-year Mazzard seedlings*

Variety	Number of grafts planted	Source of piece root	Per cent of grafts that grew	Average height of 1-year trees
				<i>In.</i>
Early Richmond	150	Collar	68	22
" "	100	Below collar	32	28
Montmorency	150	Collar	58	17
" "	100	Below collar	39	20

These results are in agreement with those of the preceding year and show that satisfactory stands may be secured by bench grafting scions on the collar piece roots.

Not shown in the table is the fact that the 1-year trees not only lacked uniformity in height but the stand was also relatively poor in comparison with the 80 per cent stand of apple grafts grown in adjacent rows. It is probable that stands would rarely exceed 60 per cent under commercial conditions. The tendency of the cherry to push its buds before planting time is an important factor in reducing the stand.

CONCLUSIONS

From these results it is evident that it is possible to secure satisfactory stands of the sour cherries Early Richmond and Montmorency when grafted on piece roots taken at the collar region of Mazzard seedlings. It may be anticipated that the percentage stand of sour-cherry grafts in commercial nurseries would be significantly less and the height would be less uniform than generally experienced with apple grafts.

From the nurseryman's point of view, a stand of grafts of approximately 50 per cent probably would be satisfactory because of the great expense involved in spraying the seedlings and the generally poor stands when sour cherries are propagated by budding on Mazzard seedlings.

From the orchardist's standpoint, there would, theoretically at least, be an advantage in planting sour cherries grafted on Mazzard seedlings because

of the resulting larger-size and longer-lived tree, in addition to the probable advantages afforded by greater protection of the rootstock from winter injury and the opportunities afforded for scion rooting.

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PHYTOPATHOLOGICAL NOTE

*A Rust-resistant Red Cedar.*¹—A common observation made during our earlier experiments with cedar-apple rust (*Gymnosporangium juniperi-virginianae* Schw.) was the marked irregularity of infection among individual cedars (*Juniperus virginiana* L.). Heavily infected trees were often found adjacent to trees with little or no infection.

In 1921 the writer began investigating the cause of this apparent difference in susceptibility. Accordingly, several trees were selected in a large cedar grove so located that all trees were subjected to relatively uniform infection. The trees were selected in pairs, one tree of each pair showing a relatively high degree of susceptibility and an adjacent one showing only slight or no infection. The trees were labeled with brass tree-markers and an annual record was made in the spring, the time of year when infection

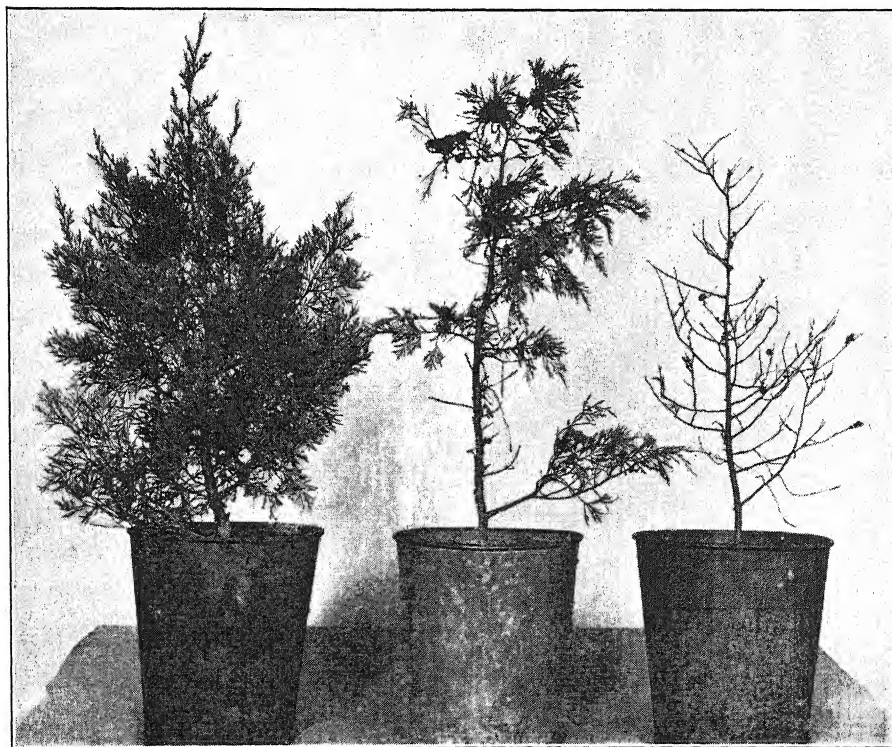
¹ Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 234.

is noticeable. A 16-year record showed, with few exceptions, that the trees selected as being very susceptible at the beginning of the experiment remained susceptible throughout the experiment, and those originally selected as resistant showed only slight infection in subsequent years.

In order to check the relative susceptibility under more closely controlled conditions, scions were taken from the most promising trees and propagated by means of grafting. Tree 16D, which in the course of 16 years had only 2 rust galls, about the size of a pea, proved to be the most resistant of the entire lot. The adjacent susceptible tree, 22X, which showed considerable annual infection throughout the experiment, was used as a check.

The trees 16D and 22X bore both adult and juvenile needles and represented the average type of cedar tree in this locality. Tree 66D was the most outstanding glaucous tree in the group, having only the adult or closely appressed needles, a type sometimes referred to by nurserymen as whipcord. This tree was moderately susceptible and was selected for propagation and comparison with the other two.

The grafted scions were grown in the nursery for a year, so that they would become well-established. They were then transplanted into the experimental apple-rust orchard, where a maximum rust infection is usually an annual occurrence.



16 D

66 D

22 X

FIG. 1. Grafted scions of *Juniperus virginiana* after 4 yrs. exposure to cedar-apple rust.

The accompanying illustration (Fig. 1) shows the results after 4 years of such exposure. The clones from the susceptible tree 22X became so heavily infected that all of them died. Those of tree 66D became severely injured, while the clones from 16D, although occasionally bearing a few galls per tree, proved to be highly resistant to the rust. It is safe to assume that under natural conditions this tree would never become severely infected with the rust prevalent in this region and would not be a menace to apple orchards.

In view of the fact that cedar rust is known to consist of several physiologic races possessing different degrees of virulence on apples² it is possible that this cedar may react differently to different races of the fungus. This problem remains to be investigated. The tree is now being propagated on a larger scale so as to make it available for distribution and for testing in different sections of the country.

As a result of the enormous losses suffered by the apple growers during the orchard expansion period, the State of West Virginia in 1912 enacted a statute which made it mandatory to destroy all cedar trees within a radius of 3 miles from any commercial orchard. Similar laws were later enacted by other States. Since the red cedar long has been the traditional evergreen of the Shenandoah Valley, such drastic legislation soon led to litigation and bitter feeling that have not completely disappeared. Aside from its purely scientific interest, a cedar tree sufficiently resistant to rust to be used for landscape purposes should, therefore, be of commercial value.

Although other species of *Gymnosporangium*, including *G. germinale* (Schw.) Kern., *G. globosum* Farl., and *G. nidus-avis* Thax., were present in the grove from which the resistant tree was selected, none was found on this tree except one very small gall caused by *G. globosum*. The propagated trees tested in the experimental apple-rust orchard were exposed to infection by *G. juniperi-virginianae* only.—ANTHONY BERG, College of Agriculture, West Virginia University, Morgantown, W. Va.

² McNew, George L. Differential reaction of apple varieties to *Gymnosporangium juniperi-virginianae*. Iowa Agr. Exp. Stat. Res. Bull. 245. 1938.

ROLAND ELISHA STONE

November 4, 1881-June 4, 1939

J. E. HOWITT

Roland Elisha Stone, Associate Professor of Botany in the Ontario Agricultural College, Guelph, died at Guelph, Canada, on June 4th, 1939, after a short illness.

He was born in Harvard, Clay County, Nebraska, on November 4, 1881. He spent his youth on the farm helping his father who was one of the early pioneer farmers of the State. Later he left the farm to enter the University of Nebraska, from which institution he graduated in 1906, having worked his way through college. In 1908 he received his M.Sc. from the Alabama Polytechnic Institute, after which he entered Cornell University and studied mycology and plant pathology under the late Dr. G. F. Atkinson. His Ph.D. degree was conferred by Cornell University in 1913. At this institution he was also elected a member of the Sigma Xi. During his residence in Ithaca, he met and was married to Miss Agnes Ready, who survives him.

In the year 1912 he was appointed Lecturer in Botany at the Ontario Agricultural College, Guelph, Canada, and, in 1917, became Associate Professor of Botany, which position he held until his death. He spent twenty-seven years in the service of the Ontario Agricultural College, Guelph.

He was a life member of the American Phytopathological Society, a member of the Canadian Phytopathological Society, the Botanical Society of America, and a Fellow of the American Association for the Advancement of Science.

Dr. Stone's time was largely taken up with teaching plant physiology and mycology. He was an excellent teacher. This was due largely to the fact that he refused to "spoon-feed." He believed that a student should not be told anything that he could readily find out for himself. The more his students knew him, the better they liked him.

He was also keenly interested in all the various student activities and up until the year of his death served as assistant coach for the senior football team. He had a quiet, unobtrusive manner and a kindly personality combined with a dry sense of humor. These qualities endeared him to all who became well acquainted with him. His outstanding characteristic, however, was his spirit of loyalty to his profession, his College, his colleagues, and his friends.

Academic work demanded much of Dr. Stone's time, but he was an enthusiastic and diligent botanist and, in addition to teaching, managed to do very considerable botanical research, specializing in plant pathology. Among his many achievements in this field are: the selection of varieties of peas resistant to root rot and suitable to the Ontario canning industry; the testing



ROLAND ELISHA STONE

of varieties of oats for resistance to loose and covered smut; and his publications on the edible and poisonous mushrooms of Ontario.

His death is a distinct loss, not only to the Ontario Agricultural College, but also to Plant Pathology in Canada.

Publications that bear Dr. Stone's name as author or co-author are:

1. The life history of *Ascochyta* on some leguminous plants. *Ann. Mycol.* 10: 564-594. 1912.
2. The smuts and rusts of grain crops. *Ont. Dept. of Agr. Bull.* 229. 1915.
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FUNGI ASSOCIATED WITH DENDROCTONUS FRONTALIS IN KILLING SHORTLEAF PINES AND THEIR EFFECT ON CONDUCTION¹

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(Accepted for publication May 7, 1940)

INTRODUCTION

Dying of pine trees infested by the bark beetle, *Dendroctonus frontalis* Zimm., has been a cause of heavy losses among pine stands in the southern United States. Infestations have occurred upon healthy vigorous pines of all species and of nearly all ages occurring within the range of the beetle. Recent studies of the biology of the beetle and of the manner in which the beetles kill living trees by Craighead (3), Nelson and Beal (9), Grossmann (4), Rumbold (10) and Nelson (8) have led to the conception that fungi, particularly the blue-staining fungus, *Ceratostomella pini* Münch, play an important part in successful infestation and killing of living trees. In connection with further studies of the physiology of infested pines, Caird (2) found that several other fungi in addition to *C. pini* were commonly associated with the bark beetle and in the sapwood of infested trees. As the relative importance of those other fungi described by Caird in comparison with *C. pini* was problematical, the present study was undertaken to aid in clearing up the general picture of fungous infection of beetle-attacked trees and to compare the effect produced by other fungi with the effect produced by *C. pini* when inoculated into healthy pines. This work was carried out at Asheville, North Carolina, during the field seasons of 1932, 1934, and 1935.

MATERIAL AND METHODS

Shortleaf pine (*Pinus echinata* Mill.), which is one of the species of southern pine commonly attacked by *Dendroctonus frontalis*, was used in all isolation and inoculation work presented in this paper.

Isolation of fungi from the wood of attacked trees to study their occurrence was accomplished through a modification of a method used by Caird (2). Complete cross-sectional disks, 1½ in. thick, were sawed at 12-in. intervals along the infested portion of the stem to be analyzed. A rectangular block, 1 in. wide and extending radially from the pith to the bark, was then cut from each disk; the disks had been previously oriented and marked so that each sample block could be taken along a direct vertical line drawn from the base to the tip of the stem. To isolate fungi from the sample blocks, the tangential face of each growth ring to be sampled was exposed successively by splitting the sample block so as not to touch the portion of the tangential

¹ Investigation by the Division of Forest Pathology, Bureau of Plant Industry, United States Department Agriculture. In cooperation with the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department Agriculture.

² Part of the investigation was carried out by the senior author under a National Research Council Fellowship in Zürich, Switzerland.

face that was to be cultured. A small piece of wood was then removed from the center of the tangential face of the growth ring and placed on a malt-agar slant in a test tube. Samples from each growth ring beginning with the exterior of the stem and proceeding inwards were taken. The culture tubes containing the wood inoculum were observed for 30 days and all visible microbial growth issuing from the wood during that period was recorded. The yeasts were checked by plating out selected cultures typical of mixed growth.

The method of inoculation used in this study was designed to give a comparison between other fungi and *Ceratostomella pini* rather than an exact comparison with natural inoculation by beetles whose complex tunneling is difficult to imitate. A simple method that could be uniformly duplicated and not result in sufficient wounding to kill the trees was devised after a series of preliminary tests. In early tests it was found necessary to place inoculation points so that they completely encircled the stem, or nearly so, in order to get successful results with *C. pini*. On the other hand, complete girdling of the stem at any one point had to be avoided because that resulted in the death of check trees by the following summer. The two methods employed, therefore, provided for complete encircling of the stem by means of alternate inoculation wounds made so as to avoid complete girdling of the stem at any one point (Fig. 1). In the method used for small trees, 2-4 in.

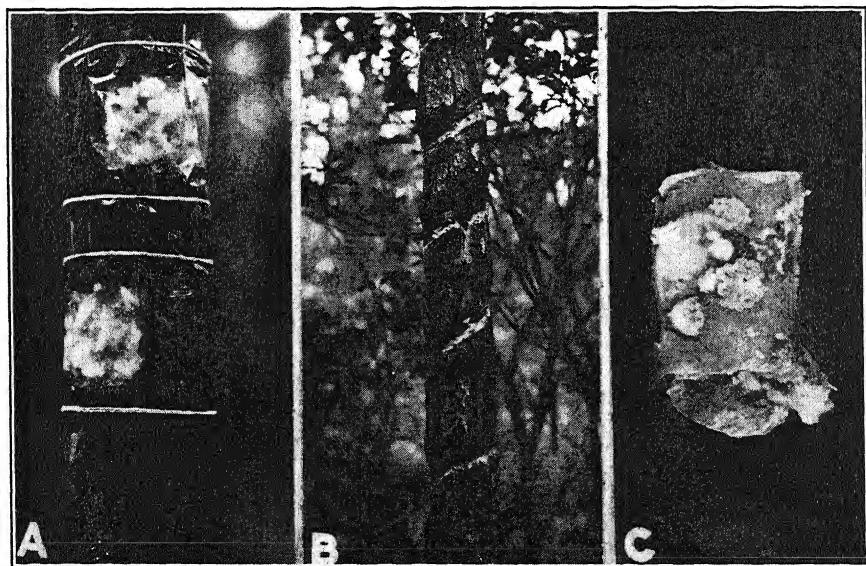


FIG. 1. A and B. Methods of inoculation used for small pines, 2 to 4 in. d.b.h., and large pines, 4 to 6 in. d.b.h., respectively. C. The gelatinous bodies formed by *Dacryomyces* sp. in culture on Lima-bean agar.

d.b.h., 2 bands of bark each $1\frac{1}{2}$ in. wide were cut so that they each slightly more than half-encircled opposite sides of the stem. The bands were spaced 2 in. apart vertically, leaving intact bark between to avoid complete girdling

at one point. For larger trees, 4-6 in. d.b.h., $\frac{3}{4}$ -in. strips each 6 in. long were cut spirally encircling the stem at an inclination of 45° with the vertical. The strips were spaced about 6 in. apart on the spiral and $1\frac{1}{2}$ in. of intact bark was left between strip ends so as not to girdle the stem. The intervals between the strip ends were not in a direct vertical line. Enough strips were cut to encircle the stem 4 times. In both methods, the bark was removed from the inoculation bands and the inoculum smeared directly on the surface of the exposed sapwood. The inoculations were covered with a cellophane strip to reduce drying. A rice culture of the fungus to be tested was used as inoculum, or, in case of the yeasts, the organisms were first grown on malt-agar slants and then mixed with sterile rice just before inoculation. Check inoculations were made with sterile rice in the same manner. Both types of inoculation wounds formed callus tissue on check trees and showed signs of healing without visibly affecting the growth or vigor of the trees during the year following inoculation.

FUNGUS INFECTION OF INFESTED PINES

Entrance of Infection

A heavy fungous infection invariably accompanies successful infestation of living pines by *Dendroctonus frontalis*. Such infection is made possible by the extensive and deep-laid tunneling of the bark by attacking beetles, the details of which have been studied and described by St. George and Beal (11). Attacking beetles bore in through the corky bark to excavate long, winding egg galleries in the underlying phloem, which may be so deep-laid as to score the surface of the sapwood. The young larvae that develop from the eggs in the phloem extend the tunneling laterally by boring their way through that tissue until full grown; they then bore outward to the corky outer bark, where they locate themselves in oval pupal chambers to become transformed to pupae. In about 7-10 days, the pupae make the final change to adults and the insect bores outward to emerge through exit holes in the bark. Under favorable conditions, beetles may complete their development from egg to adult in about 40 days.

TABLE 1.—Isolation of organisms from various stages of *Dendroctonus frontalis* infesting shortleaf pine

Insect stage	Number trees cultured	Number insects cultured	Organisms appearing in culture							
			<i>C. pini</i>		<i>Dacryomyces</i> sp.		<i>Z. pini</i>		<i>Monilia</i>	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Adult	10	73	30	41	13	18	39	53	20	27
Eggs	14	1	7	2	14	9	64	4	28
Larvae	25	0	0	8	32	13	52	0	0

The characteristic thoroughness with which bark may be tunneled by attacking beetles is of particular importance to a consideration of fungous infection, for it is these numerous tunnels that permit an early and heavy infection of both the inner bark and sapwood of infected trees. As shown in table 1, certain organisms that were later found in the sapwood during early stages of attack were found on and in the bodies of beetles, and, presumably, the thoroughness of infection may be ascribed to direct inoculation with fungi carried by beetles, as well as through chance entrance of wind-blown spores. The cultures were made by placing the insects on agar slants with a sterile needle or brush.

Fungi that Commonly Infect the Sapwood

Primary infection of the sapwood of infested pines was not a simple infection by *Ceratostomella pini*, alone, but proved to be a complex infection by several fungi that entered the wood more or less simultaneously. While this complexity held true for both early and late stages of infestation, and confirmed evidence of a similar nature presented by Caird (2), the earliest or primary infection was typically affected by a group of specific fungi constantly found in the sapwood during the entire study.

A summary of the data obtained from analyses of 12 infected shortleaf pines of from 4-6 in. d.b.h. representing successive stages of infestation by *Dendroctonus* indicates the complex fungous population of infested trees (Table 2). Most of the trees were infested by *Ips* spp. in the tops above the *Dendroctonus* when collected during the last two stages of infestation. These trees were collected from 3 separate localities in the Pisgah National Forest near Asheville, North Carolina, during the growing seasons of 1932, 1934, and 1935. The fungi included in that table are those constantly isolated from the sapwood of the portion of the trees infested by *Dendroctonus*, and may be briefly described for reference in connection with this paper as follows:

Ceratostomella pini Münch.—An ascomycete causing blue stain of wood. Previously described in connection with *Dendroctonus frontalis* by Rumbold (10) and Nelson (8).

Dacryomyces sp.—Identity of this fungus has not been definitely established, but it has been tentatively assigned to *Dacryomyces* on the basis of a similarity in growth form and asexual-spore formation. The following characteristics have been used to identify the fungus when grown on potato-dextrose agar in culture tubes: sparse grayish-white to brown aerial mycelium appears in young cultures and the agar turns brown near the surface. The mycelium usually has prominent clamp connections, but these structures may be lacking or may disappear upon repeated transferal of the fungus from one culture tube to another. As the aerial mycelium becomes more abundant, forming a compact mat over the surface of the agar, a brown exudate is commonly found attached to the sides of submerged hyphae. The agar finally becomes completely discolored and the mycelium slowly

TABLE 2.—Organisms isolated from sapwood of twelve shortleaf pines 18–20 years old, infested by *Dendroctonus frontalis*. Five trees were sampled first two stages and two trees for 15–30 day stage. Only the infested portions of the stem were cultured

Age of attack	Organism	Number of cultures per growth ring ^a																Total no.	Cultures, per cent
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
-7 days	None	24	31	39	41	41	28	9										213	72
	<i>Ceratostomella pini</i>	4	1															5	2
	<i>Dacryomyces</i> sp.	13	11	9	4	3												40	13
	<i>Zygosaccharomyces pini</i> ..	2	1															3	1
	<i>Monilia</i> spp.																	13	4
	<i>Trichoderma lignorum</i>																	0	0
	Bacteria	4	3															7	2
	<i>Penicillium</i> spp.	5	3		1													11	4
	<i>Ceratostomella ips</i>																	0	0
	Misc. fungi	3	2															5	2
-14 days	Total cultures	63	56	50	47	44	28	9										297	
	None	25	27	30	35	36	35	32	40	31	28	15	10	5	2			351	39
	<i>C. pini</i>	9	10	10	13	9	9	6	6	3	4	2	1	2				84	9
	<i>Dacryomyces</i> sp.	14	18	19	16	19	20	24	20	22	13	7	8	4				204	23
	<i>Z. pini</i>	21	17	13	9	7	6	2	1	1			2	1	1			83	9
	<i>Monilia</i> spp.	9	9	9	8	5	6	5	5	7	5	3	2	1				74	8
	<i>T. lignorum</i>	2	3	3	4	3	1	1	1	1	1		2					17	2
	<i>Penicillium</i> spp.	3	1	2	2	1	2	3	4									18	2
	Bacteria	5	4	3	3	1	1	1	1									19	2
	<i>C. ips</i>	4	3	2	1	1	1	1	1									13	2
-30 days	Misc. fungi	7	7	4	3	1	2	2	2									28	3
	Total cultures	99	99	95	94	83	83	76	80	64	50	29	23	13	3			891	
	None	1	1	1	1	1	3	4	6	3	4	5	4	2				36	7
	<i>C. pini</i>	12	15	14	14	14	12	12	10	10	8	9	6	6	2	1		145	30
	<i>Dacryomyces</i> sp.				1		1					2	1					5	1
	<i>Z. pini</i>																	2	1
	<i>Monilia</i> spp.	8	8	9	10	11	10	10	9	10	10	7	7	6	3	1	1	120	25
	<i>T. lignorum</i>	13	9	11	11	9	7	5	4	5	5	3	2	1	1			86	18
	<i>Penicillium</i> spp.																	3	1
	Bacteria	13	10	8	8	6	4	7	4	4	1				2			67	14
-30 days	<i>C. ips</i>	1	1	1	1	1	2	1	1	1	1							8	2
	Misc. fungi	1	1				1	1	1	1	1	1	2	15	6	4	2	9	2
-30 days	Total cultures	49	45	45	46	42	40	39	35	33	32	26	22	15	6	4	2	481	

^a Numbered successively beginning with outermost ring of sapwood as number 1 and proceeding inwards to heartwood.

turns from gray to buff. Chlamydospore-like cells are found at the tips of submerged hyphae and at intermediate positions along their length in older cultures. Small, convolutely folded, gelatinous bodies are later developed in culture both on agar and on pine wood. These bodies have not been observed to produce spores, but chlamydospores have been produced on flat stromata on pine wood in culture.

Zygosaccharomyces pini Holst.—A new species of yeast commonly found in association with *Dendroctonus frontalis*, recently described by Holst (5).

Monilia spp.—A group of anascosporogenous, mycelium-forming yeasts belonging to the *Mycotoruloides*.

Trichoderma lignorum.—A species of green mold commonly found in the sapwood of infected trees during late stages of infestation.

Bacteria.—Several unidentified types were isolated, the most common of which was a gram-negative rod, nonmotile, which occurred singly and in pairs.

Penicillium spp.—Several species were found in the wood and coming into cultures as laboratory contaminants.

Ceratostomella ips Rumbold.—An ascomycete causing blue stain of wood, described by Rumbold (10) as being constantly associated with bark beetles of the genus *Ips*.

Miscellaneous Fungi.—Unidentified fungi, probably wood-decaying saprophytes that appeared in culture as white or buff cottony mycelium without spores or fruiting structures.

An interesting feature of the relative abundance of various species of fungi in the sapwood of attacked trees at different stages of infestation shown in table 2 is the decided change in fungus population that occurs proceeding from one stage of infestation to another; both the species present and their relative abundance undergo marked variation according to the length of time following initial attack by the beetles.

For convenience in describing the progress of fungous penetration, the period of infestation has been divided into 3 stages. The first stage includes the time period, 1–7 days after initial attack by the beetles. During that time initial invasion of the sapwood takes place, the foliage of the attacked tree remaining green. The second stage includes the period, 8–14 days after attack, when the foliage begins to turn yellow; some fungi reach the heartwood of stems 4–6 in. in diameter by the end of this period. The third stage includes the period, 15–30 days after attack, during which time the foliage turns from yellow to reddish-brown.

Dacryomyces sp. is the most common fungus during first 1–14 days, and then, appears rarely in culture during late stages when *Ceratostomella pini*, *Monilia* spp., and *Trichoderma lignorum* reach their maximum abundance in the sapwood. It is possible that the presence of *Dacryomyces* sp. in wood inoculum may be masked by *C. pini* or other fungi which grow more rapidly in culture. *C. pini* is not abundant during the early stages of infestation, but becomes the most commonly occurring fungus during late stages.

Monilia spp. and *T. lignorum* are sparse during early stages. These and other variations indicate that the time, or depth, of examination is an important feature in the study of fungi in beetle-attacked trees. From those results it may be readily understood why *C. pini* has been of major interest in previous work, which began with observations on trees in late stages of infestation, because at that time *C. pini* not only becomes abundant in the sapwood but also obscures other organisms. The fungus population during late stages in infestation when the foliage exhibits visible symptoms of dying, however, is not considered to be as important as when the initial infection of the sapwood takes place and before visible color change has occurred in the foliage, i.e., during the first 2 weeks of infestation. It is during the periods 1-7 days and 8-14 days after attack that complete primary infection of the sapwood takes place and stoppage of conduction is first noted in the sapwood. The fungi infecting the sapwood during the first 14 days after attack, therefore, have been considered of most importance to this particular study.

Comparative Rate of Fungous Penetration into the Sapwood

The primary invading fungi enter the sapwood soon after infestation and tunneling of the bark by the beetles. These fungi may enter the sapwood directly, as the beetle tunnels usually score the surface of that tissue, or they may first infect bark tissue surrounding the tunnels and proceed from that tissue into the underlying sapwood. According to cultures obtained from a pine on the evening of the first day of attack, infection may take place within 24 hours after the initial attack by beetles. Cultures taken from the phloem surrounding the beetle tunnels and from underlying sapwood to a depth of about 1 mm. (1-2 growth rings) yielded *Dacryomyces* sp., *Ceratostomella pini*, and *Zygosaccharomyces pini*; elsewhere the sapwood and phloem were sterile. Such early penetration indicates that infection precedes any considerable drying of the sapwood through loss of moisture from beetle tunnels, but does not preclude the possibility that a certain slight drop in moisture may be necessary to penetration into the outer growth rings. The ability of fungi to penetrate into the sapwood shortly after initial entrance by beetles is perhaps a significant feature in connection with their effect on the attacked tree.

After the fungi have successfully entered the sapwood, they penetrate rather rapidly towards the center of the stem to reach the heartwood of trees 4-6 in. d.b.h. within 14 days. Their progressive penetration is accompanied by cessation of water conduction to the leaves and gradual drying of the sapwood. The general situation during the earlier stages of infestation, 1-7 days after attack, has been diagrammed as shown in figure 2, using maximum fungous penetration from table 2. The primary invading fungi have penetrated to unequal depths during this period. *Dacryomyces* sp. has penetrated more deeply into the sapwood than the other fungi; *Ceratostomella pini* and *Zygosaccharomyces pini* lag somewhat behind the *Dacryo-*

myces; while both *Monilia* spp. and *T. lignorum* have not yet entered the sapwood. Penetration shown for *Penicillium* cannot be taken as significant, because that fungus occurred as a common laboratory contaminant and appeared in widely separated cultures. Bacteria and miscellaneous fungi are present in the outer rings only.

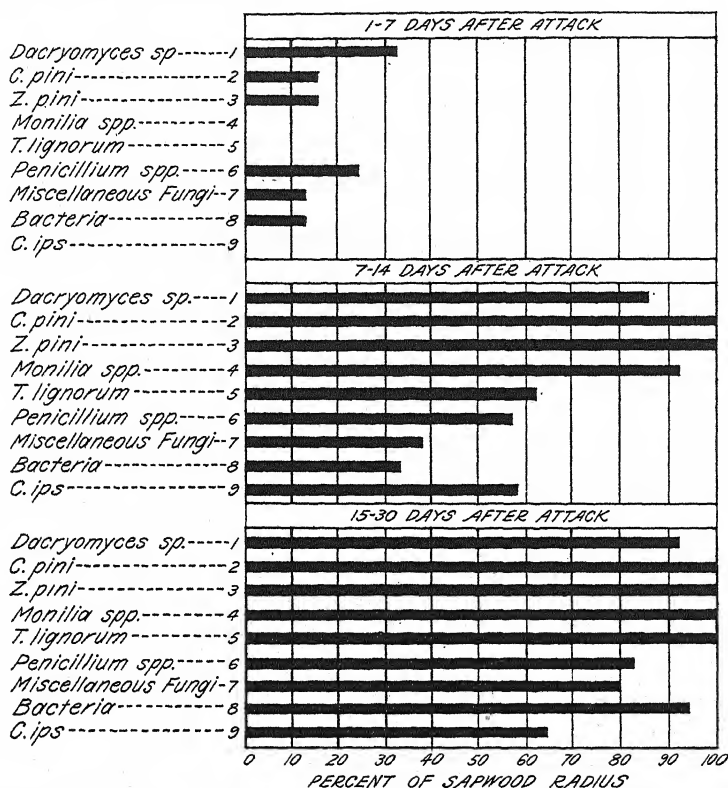


FIG. 2. Maximum fungous penetration into shortleaf pines during various stages of infestation. The percentage figures represent comparative depth of penetration into the sapwood, 100 per cent being penetration to the heartwood. Five trees were sampled for the first two stages and two trees for the 15-30-day stage. The trees were collected at three separate localities in the Pisgah National Forest.

The distribution and penetration into the sapwood of the three most prominent organisms during the period 1-7 days after attack are shown by bar diagrams in figure 3. Those organisms were selected for separate presentation because of their invariable presence as primary invaders of the sapwood during early stages of infestation. *Dacryomyces* sp. has not only penetrated more deeply than the other fungi, but it has also been isolated from every disk between 1 foot and 11 feet above the stump. Maximum penetration by this fungus has occurred at about the center of the attacked portion of the stem. *Ceratostomella pini* was present during this period, although the sapwood showed no indication of blue stain. *Zygosaccharomyces pini* was isolated from the outer rings only.

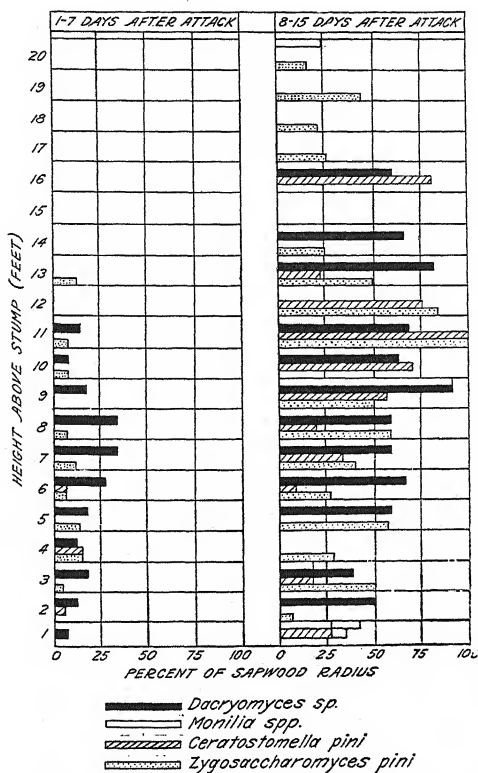


FIG. 3. Maximum penetration by fungi according to height above the base beginning with samples taken at 12 inches and extending upwards along infested portions of the stems. Five trees were sampled for each period.

During the second period of infestation, 8-14 days after attack, some fungi reach the center of the stem (Fig. 2). The relative depth of penetration changes as *Ceratostomella pini* and *Zygosaccharomyces pini* advance more rapidly than *Dacryomyces* sp. and reach the center of the stem before the latter. *Monilia* spp. and *Trichoderma lignorum* enter the sapwood during this period, and are evidently able to penetrate closely behind the primary invaders. Other fungi are restricted to the outer layers of sapwood.

Distribution of the 3 primary invaders previously shown for the 1-7-day period is shown also for the 8-14-day period in figure 3 with the addition of *Monilia* spp. *Dacryomyces* sp. is still most prominent, although its depth of penetration has been exceeded by that of *Ceratostomella pini*, which has reached the center of the stem. Prominent blue-stain wedges have appeared in the sapwood during this period. *Zygosaccharomyces pini* occurs sporadically over the attacked portion of the stem and has also reached the innermost layers of sapwood. During this stage *Monilia* spp. have become common in the sapwood and have penetrated deeply.

Distribution of fungi during the late period, 15-30 days after attack, changes somewhat as the secondary invaders continue to penetrate into the

sapwood and often reach the innermost layers (Fig. 2). It seems probable, however, that distribution during this period is not significant in relation to effect of fungi on conduction, because the sapwood already has become infected as far as the heartwood, and has ceased to conduct water to the leaves. *Trichoderma lignorum* becomes prominent in the sapwood during this period. The wood is heavily stained through accumulation of hyphae of *Ceratostomella pini*, and also, in some cases, of *C. ips*.

INOCULATION OF HEALTHY PINES WITH FUNGI ISOLATED FROM BEETLE-INFESTED PINES

The invariable occurrence of fungi in the sapwood of infested trees and the penetration of these organisms to the center of the stems during early stages of infestation lend support to the view that fungi play an active part in bringing about changes in the sapwood that stop upward conduction of water through the stems and cause death of the crowns. Such a role has been assigned specifically to *Ceratostomella pini* by Nelson (8), who proposed that fungous infection affects the tori of wood tracheids to bring about stoppage of conduction and to cause infested trees to die more rapidly than could be effected through girdling by beetle tunnels. From a somewhat different viewpoint, Caird (2) has suggested that fungi may act internally to accelerate drying of the sapwood of infested trees. Both of the preceding investigators succeeded in causing death of living pines by means of inoculations with *C. pini*, while a second fungus, *Trichoderma lignorum*, which was commonly isolated from the sapwood of infested trees, did not measurably affect living pines. The primary purpose of the inoculation results presented in this paper is to compare the effect of other primary invading fungi with *C. pini*, in order to ascertain whether or not the action of *C. pini* described by Nelson would be duplicated by other organisms. From results presented earlier in this paper, there seems to be little doubt that several other fungi besides *C. pini* may be isolated from the sapwood of infested pines shortly after they have been attacked by beetles. The question that remains to be answered is, "can these other fungi also penetrate into the sapwood of healthy pines following inoculation and bring about the death of those trees?"

Effect of Inoculations

Results of inoculations made with individual fungi in June and July of 1933 and 1934 are summarized in table 3. *Ceratostomella pini* was the only fungus able to cause the death of trees of from 2 to 6 inches diameter breast high. Although not all of the inoculated trees died, the results obtained from *C. pini* may be considered significant when compared to check trees that were treated similarly in all respects excepting the introduction of *C. pini* into the inoculation wounds and with trees inoculated with other fungi. *C. pini* was reisolated as the primary invading fungus from all inoculated trees. One of the main causes of variation in the success of

C. pini inoculations was the failure to secure successful inoculations on all sides of the stem. As has been pointed out by Nelson, the death of inoculated trees occurred only when successful inoculation was secured on all sides of the tree; if a continuous band of sapwood remained noninfected the tree continued to live. In one case during the present study, which may be taken as an example, a tree of 4-6 in. in diameter at breast height was living and apparently healthy 12 months after inoculation with *C. pini* owing to an uninterrupted streak of uninfested sapwood about 2 in. in width that ran longitudinally through the inoculation bands. *C. pini* is evidently able to penetrate tangentially but a short distance under intact healthy bark. A series of trees, 2-4 in. d.b.h., which were inoculated in 1932, further illustrate this characteristic. The inoculations were made on each tree by removing a band of bark, $1\frac{1}{2}$ in. wide and half-encircling the stem. *C. pini* was applied by smearing the surface of the exposed sapwood with a vigorous

TABLE 3.—Inoculations of living shortleaf pines with fungi isolated from the sapwood of pines infested with *Dendroctonus frontalis*. All inoculations were made during June and July and were observed for the remainder of the growing season, a period of from 2-4 months

Organism used	Trees inoculated		Trees dying	
	Number	Diameter breast high (inches)	Number	Months after inoculation
<i>Ceratostomella pini</i>	12	1-2	12	$1\frac{1}{2}$
	12	2-4	6	$1\frac{1}{2}$
	12	4-6	8	1
			2	4
<i>Dacryomyces</i> sp.	12	1-2	3	1
	12	2-4	1	$\frac{1}{2}$ ^a
	6	4-6	0	4
<i>Zygosaccharomyces pini</i> ...	6	1-2	0	4
	6	2-4	0	4
<i>Monilia</i> spp.	18	2-4	0	4
<i>Trichoderma lignorum</i>	12	1-2	0	4
	6	2-4	0	4
	3	4-6	0	4
Sterile rice	6	1-2	0	4
	6	2-4	0	4
	9	4-6	0	4

^a *Ceratostomella pini* was isolated from the sapwood of this tree.

rice culture of that fungus. The fungus penetrated to the center of the stem under the inoculation wound and infected half of the cross-section of the sapwood; the other half of the sapwood under intact bark remained uninfected. These trees were living 2 years after inoculation, during which time the fungus failed to extend tangentially to infect the sapwood lying under intact bark.

Results from inoculation tests with *Dacryomyces* sp. indicated that it is not able to kill trees that are larger than 1-2 in. in diameter breast high. In one case in which a tree above 2 in. d.b.h. died following inoculation, subsequent examination and culturing of the sapwood showed that *Ceratostomella pini* was present as a contamination. Trees ranging in size from 4 to 6 in. d.b.h. were not killed, although the fungus was reisolated later from the sapwood of outer growth rings, where it had remained viable but unable to penetrate further into the sapwood. Apparently, this fungus is incapable of penetrating more than a few mm. into the sapwood of a living tree over 2 in. d.b.h.

The other organisms used in these inoculation tests, namely, *Zygosaccharomyces pini*, *Monilia* spp., and *Trichoderma lignorum*, proved ineffective when inoculated into healthy pines. These organisms failed to penetrate into the sapwood beyond the growth layers dried as a result of exposure through inoculation wounds. Several cultural growth forms of each organism were tried, including cultures isolated from infested trees in different years and rough and smooth strains of *Monilia*.

Effect of Combined Inoculations

As the fungi infesting beetle-attacked trees have been found growing together in close association in the sapwood, the question has been raised as to whether certain of those fungi may not act together to injure the tree. It was thought possible that one fungus, which alone may not have been able to penetrate into the stem to injure the tree, might act in cooperation with a second fungus to produce an active combination. It also seemed possible that one fungus might have an antagonistic or toxic effect on other fungi, thus reducing their harmful effects or causing their elimination from the living population of the sapwood during late stages of infection. Of the possible combinations of fungi that might be tested for interaction the most promising was a combination of *Zygosaccharomyces pini* with *Ceratostomella pini* where the yeast might act to stimulate the fungus, and the combination of *Trichoderma lignorum* with *C. pini* where the first-named fungus might act to destroy *C. pini* in a manner in which it has been reported to act in relation to certain soil fungi (1).

To seek an answer to the above questions, the fungi found in the sapwood were combined in various ways and healthy trees were inoculated with these combinations in the same manner as previously used for single fungi. In addition to inoculations with combinations of fungi, successive inoculations were made with two or more fungi at one point, for example, inoculations were made with pure culture of *Zygosaccharomyces pini* and after a short period inoculates from pure cultures of *Ceratostomella pini* were applied to the same inoculation wounds.

The results of inoculations with combinations of fungi are presented in table 4. A comparison of the data presented there with data on inoculations with single fungi indicate that, under the conditions of inoculation

and methods employed, combinations of fungi were no more effective than inoculations with single fungi. Combinations were effective in killing trees only when *Ceratostomella pini* was used and no marked increase in virulence was noted over that obtained when *C. pini* was used alone. Results obtained the year previous with a combination of *Zygosaccharomyces pini* with *C. pini* had given more consistent results than when *C. pini* was used alone, but upon repetition the difference was found to be insignificant. A possible retarding effect exerted by *Trichoderma lignorum* upon *C. pini* was observed but was not sufficiently tested to be conclusive.

TABLE 4.—Inoculation of healthy shortleaf pines with combinations of fungi isolated from *Dendroctonus frontalis* infested pines

Combination of inoculating fungi ^a	Trees inoculated ^b		Trees dying	
	Number	Diameter breast high (inches)	Number	Days after inoculation
<i>Ceratostomella pini</i>	6	2-4	4	26
<i>Ceratostomella pini</i> + <i>Zygosaccharomyces pini</i>	6	4-6	5	40
	6	2-6	6	45
	6	2-4	4	26
<i>Ceratostomella pini</i> + <i>Monilia</i>	6	2-4	2	28
<i>Ceratostomella pini</i> - <i>Trichoderma</i> ..	6	2-4	0	60
<i>Zygosaccharomyces pini</i> - <i>Ceratostomella pini</i>	6	4-6	0	60
<i>Zygosaccharomyces pini</i> + <i>Dacryomyces</i> sp.	6	2-4	0	60
	6	1-2	0	60
	6	2-4	0	60
<i>Dacryomyces</i> sp. - <i>Ceratostomella pini</i>	6	2-4	0	60
<i>Dacryomyces</i> sp. - <i>Trichoderma</i>	6		0	60
<i>Zygosaccharomyces pini</i> - <i>Dacryomyces</i> sp. - <i>Ceratostomella pini</i>	6	2-4	0	60
<i>Trichoderma</i> - <i>Ceratostomella pini</i> ..	6	2-4	0	60

^a - followed by; and + added to.

^b For inoculations made at the same time with sterile rice, see table 3.

Effect of Inoculations on Conduction

A comparison of the effect of inoculations on conduction of water through the sapwood to the crown was obtained for the most promising of the fungi isolated from infested trees by means of a study of dye patterns from inoculated and noninoculated pines. This study gave a more complete comparison of penetration and subsequent effect on the sapwood than did observations on dying of crowns, as the latter gave information only on those fungi that caused complete stoppage of conduction.

The dye patterns were obtained by the following method: Trees to be studied were girdled at the base on the day prior to their examination by cutting through the outer $\frac{1}{4}$ in. of sapwood to stimulate resin flow. Such treatment served to exhaust the immediate resin flow of the trees and eliminate an excessive resin flow that otherwise made intake of dye solution by severed stems very irregular. On the following day, the trees were severed at their bases above the girdle and the severed end placed at once in an aqueous solution of light green F.S. (1:2000). The stems were examined after 24 hours and the dye patterns at 30–36 in. above base were diagrammed in cross section. Refinements of the above method designed to prevent entrance of air into the stem were tried and found to be unnecessary to obtain complete dye patterns for stems of healthy shortleaf pines. The chief source of variation was due to excessive resin flow from cut ends, and this was eliminated by the shallow girdling described above.

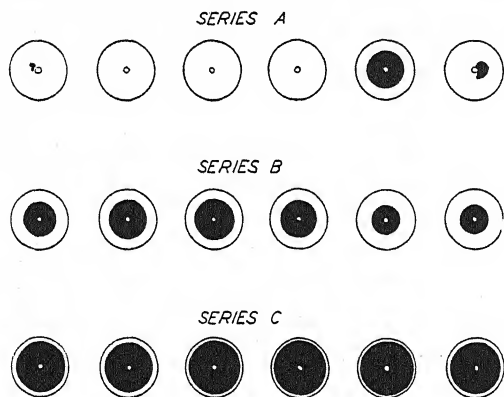


FIG. 4. Diagrams of actual conduction patterns obtained from the center of inoculated portions of stems taken 2–4 months following inoculation. Shaded areas represent portion of sapwood conducting dye. Series A, six trees inoculated with *C. pini*. Series B, six trees inoculated with *Dacryomyces* sp. Series C, six check trees inoculated with sterile rice.

The type of dye patterns obtained from inoculated stems, 2–4 inches d.b.h., as compared to healthy uninoculated stems are presented in figure 4. Stems inoculated with *Ceratostomella pini* were the only ones that became unable to conduct dye through the sapwood to the crown within a 2-month period. *C. pini* was invariably reisolated from the non-conducting sapwood under inoculations. The dye patterns shown by stems inoculated with *Dacryomyces* sp. indicated that conduction was not stopped in the inner layers of sapwood; the fungus was isolated only from the outer non-conducting sapwood. Inoculations with *Zygosaccharomyces pini* and *Monilia* spp. did not affect dye conduction through the sapwood. The outer few mm. of nonconducting sapwood shown in the latter inoculations was presumably due to wounding and external drying of sapwood exposed by the inoculation wounds as it was similar to the nonconduction shown by healthy check trees inoculated with sterile rice. Failure of *Dacryomyces* sp. and *Z. pini* to

penetrate into the sapwood further than the external growth rings which had been exposed to drying may be caused by an inability to grow into moist wood when the oxygen supply is low, or, possibly by an inability to parasitize living cells of wood. *C. pini*, on the other hand, appears capable of penetrating sapwood to considerable depths, where that wood is exposed externally by removing the bark. According to Lagerburg, Lundberg, and Melin (6) *C. pini* can grow in wood that dries slightly (2-3 per cent) to a moisture content of 171 to 175 per cent based on dry weight, provided it has an immediate access to air at the surface of the wood. Sapwood of the outer growth rings of shortleaf pines (2) averages about 110 to 175 per cent moisture on a dry weight basis, so that with some drying due to exposure when stems are inoculated, *C. pini* should be able to grow in the wood.

A dye pattern similar to that obtained from the 2-4-in. trees was obtained when 4-6-in. trees were inoculated with *Ceratostomella pini*, *Zygosaccharomyces pini* and *Dacryomyces* sp., respectively. Trees inoculated with *C. pini* invariably showed an interference with conduction amounting to complete stoppage in nearly all cases, while trees inoculated with *Dacryomyces* sp. showed an interference with conduction of dye through the outer rings only. Trees inoculated with *Z. pini* gave dye patterns similar to check trees inoculated with sterile rice.

Stoppage of Conduction by *Ceratostomella pini*

The manner in which *Ceratostomella pini* acts to bring about stoppage of conduction through the stems of infected trees has not been the subject of much direct experimentation, although some interesting theories have been formulated in connection with that fundamental phase of the blue-stain bark beetle problem. Nelson (8) has proposed that permanent aspiration of tori, following occlusion of apertures in the membranes of bordered pits by decomposition products of fungi or protoplasmic residues from attacked ray parenchyma cells, stops the transpiration stream. This theory seems to be logical in light of observations made, but should be more critically examined and tested before it is accepted as final. Conclusive demonstration of mechanical blockage seems to be lacking and the substances supposed to clog the pit apertures have not been clearly shown to be present.

As a first step in a study of the cause of stoppage of conduction, tests have been made to determine whether or not passage of water through tracheids of the sapwood becomes blocked by substances which solidly close these conducting elements, or whether the stoppage is due to trapped air or gases. The experiments presented in this report do little more than initiate the study, but have produced some interesting results that seem worthy of mention.

The experimental setup was designed to test the stoppage of conducting elements in sections of stems infected by *Ceratostomella pini* as compared to sections from noninfected stems exposed to air drying or dried by transpiring crowns. The inoculations consisted of removing a 1-in. band of bark

encircling the stem and applying fresh *C. pini* cultures as inoculum to the exposed sapwood. Check trees were similarly treated without inoculation. As soon as the foliage began to turn color, the inoculated trees were tested for stoppage of conduction by forcing a light green F.S. dye solution through 3-in. stem sections taken 1 in. apart along the stem with the inoculation on the middle section. The pressure used to force the dye solution through the stems was obtained from a 25-mm. head produced by attaching a rubber tubing to one end of the stem section to be tested and suspending the tubing and stem in a perpendicular position.

The first tests were made on noninfected trees to determine whether air sucked into stems as they were dried through withdrawal of water by transpiring foliage would block dye conduction through stem sections. Tests on 2 noninfected stems showed that dye passed through the outer sapwood, even when dried to a point at which free water no longer existed in the tracheids. When these were compared with 3 noninfected, freshly cut stems, little difference was found, indicating that drying and entrance of air into the stems had not blocked the passage of dye. Two similar stems were then cut and sections from them dried at 70° C. for 48 hours. Dye could not be forced through these oven-dried sections by pressure or by suction from a water pump. Subsequent microscopical examination of the oven-dried pieces revealed an unidentified foreign substance that took a blue stain with aniline blue dye filling the tracheids.

Tests were then made on dye conduction through 4 inoculated stems. A complete stoppage of dye conduction occurred under the inoculation bands, and, in a few cases, above the inoculated sections, as the trees died. Four noninoculated stems showed a partial stoppage of conduction in the outer rings under the girdles, which was presumably caused by wounding and exposure of the sapwood. The fungus was evidently capable of penetrating into the stems beyond outer dried sapwood to completely stop conduction. This stoppage was presumably not caused merely by entrance of air into the sapwood, as the earlier tests with air-dry stems showed no stoppage of dye. Although the exact cause of mechanical stoppage has not been definitely determined, preliminary observations have shown globules of resin present in the lumina of tracheids. These globules were absent from the wood of stems dried by transpiration and were presumably formed and released in infected sapwood owing to internal wounding by fungous hyphae. It seems possible that this resin acted to block the pit passages to passage of water and dye solutions.

EFFECT OF TEMPERATURE ON THE GROWTH RATE OF *CERATOSTOMELLA PINI*,
DACRYOMYCES SP. AND *ZYGOSACCHAROMYCES PINI*

A comparison of the growth of some of the more prominent invading organisms at various temperatures was made in the laboratory to determine the optimum temperature for each organism. Although the results of such tests seemingly do not apply directly to an explanation of variations in rate

of penetration into living stems of pines, the results have been useful in growing the organisms in culture for various purposes.

Data from growth tests on malt agar in Kolle flasks have been summarized for comparison in the form of growth curves (Fig. 5). As *Dacryomyces* sp. lagged behind *Ceratostomella pini* at all temperatures and exhib-

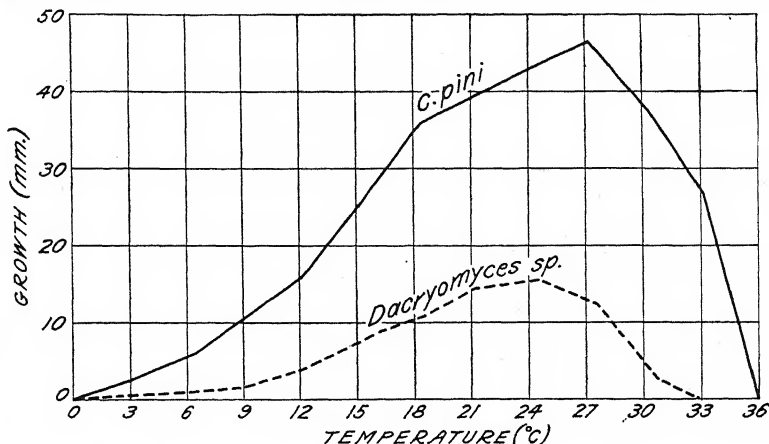


FIG. 5. Comparison between growth rate of *Ceratostomella pini* and *Dacryomyces* sp. on malt agar in Kolle flasks. The growth figure used for each temperature was the average of the 4 radii of 10 cultures taken at the end of a 5-day period.

ited a slow initial growth, the results do not aid in understanding penetration into living stems. The explanation of the latter seems to lie more in a differing ability either to grow into wood of high moisture and low oxygen content or to penetrate living tissue.

When the 2 fungi were grown on malt broth in culture flasks, the same optimum temperature was obtained for each as when grown on malt agar, i.e., approximately 27° C. for *Ceratostomella pini* and 24° C. for *Dacryomyces* sp. The yeast, *Zygosaccharomyces pini*, made its most rapid growth in malt broth at 30.6° C., a considerably higher optimum than either of the other fungi.

DISCUSSION AND SUMMARY

Fungous infection of the sapwood which followed infestation of living pines by *Dendroctonus frontalis* was not a simple infection by a single fungus but a complex invasion accomplished within a short period by a number of fungi. Certain of the fungi played an active role in aiding the beetles in bringing about the death of infested trees and have been called primary invading fungi. These were few in number and were constantly present in infested trees. They penetrated into the sapwood in advance of secondary invaders to reach the heartwood of trees 4-6 inches in diameter at breast height in about 2 weeks' time. As the sapwood became infected, water conduction through the stem to the crown ceased, indicating some interference with the transpiration stream.

Contrary to expectations, *Ceratostomella pini* was not the most prominent invader of the outer sapwood immediately following infestation, but was accompanied and even preceded in early stages by other fungi. Most prominent among these other invaders were *Dacryomyces* sp. and *Zygosaccharomyces pini*. Later, however, *C. pini* penetrated rapidly into the inner growth rings and seemed to take the lead in further infection of the sapwood. Correlated with the last observation were the results of inoculations that showed the two organisms, *Dacryomyces* sp. and *Z. pini*, could penetrate but a short distance alone into the sapwood of healthy trees, the infected tissue being within the external area affected by removal of the bark, while *C. pini* alone was able to penetrate to the center of inoculated stems of healthy trees. It is not surprising, therefore, to find *C. pini* taking the lead in penetrating deeply into the sapwood of stems wounded by bark beetles.

Distinction has been made between fungi which typically entered the sapwood immediately following infestation, the primary invading fungi referred to above, and fungi that entered during later stages when the crowns were turning yellow and that were entirely absent or scarce in the sapwood during early stages of infestation. This was deemed important, as interference with conduction occurs during initial penetration prior to color change of the foliage. These secondary invaders, or fungi appearing solely in late stages when the foliage was turning, were not considered to be of importance in connection with death of the trees. The possibility of some of the secondary invaders such as *Trichoderma* and *Monilia* being of importance in spite of their scarcity during early stages was checked by individual inoculations of healthy trees and gave negative results. Combinations of these fungi also failed to produce aggressive invasion of sapwood. It seems probable, therefore, that they merely accompany or follow primary invading fungi such as *Ceratostomella pini*.

That none of these fungi play more than an assisting role in killing the trees is evident, for even the most active fungus, *Ceratostomella pini*, requires wounds of considerable tangential extent before it can seriously damage a healthy stem. This fungus, however, has been shown by several investigators (2, 8) to be capable of killing healthy trees following wounding. Inoculation tests made during the present study have further demonstrated that when *C. pini* is introduced into trees through inoculation wounds that alone are not sufficient to cause death, subsequent growth of the fungus into the sapwood will cause the death of the inoculated tree. It has also been shown that tangential penetration by *C. pini* does not take place to any considerable extent under healthy bark, so that successful killing by *C. pini* requires wounding of the tree sufficient to permit the fungus to penetrate on all sides of the stem. An inoculation with *C. pini* that is restricted to one side of the stem will not cause the death of trees larger than 2 inches in diameter at breast height. The numerous tunnels of the attacking bark beetles furnish the necessary points of entrance in case of insect infestations; while small staggered inoculation wounds are capable of bringing about the same result in case of artificial inoculation in the absence of the

beetles. The inoculation wounds may be of a type that, alone, do not markedly reduce the apparent health of the foliage.

The chief visible effect of fungi accompanying beetle attacks on the trees is to bring about stoppage of conduction and death of the trees more rapidly than would be accomplished through mechanical injury to the stem by the beetle alone. The beetles open the stem to infection by means of numerous tunnels and even introduce fungi into the cambial region on and in their bodies. Although the drying of the stem that occurs through these tunnels is slight, such drying may be of decided importance in connection with entrance of air to growth of invading fungi. Even *Ceratostomella pini* seems to require an immediate access to air for successful growth (Münch, 7). As the fungi grow in the sapwood under the tunnels, they bring about stoppage of conduction and subsequent drying of stem, which may be important to the development of bark beetle broods (Nelson, 8).

The question as to exactly how these fungi bring about a stoppage of water conduction in the stem has not been completely answered. Nelson (8) has proposed that aspiration of tori in the pits of water-conducting tracheids stops the transpiration stream. He suggests that decomposition products or protoplasmic residue might plug the perforations in the membranes of bordered pits and tension from the water column bring on permanent aspiration. Preliminary experiments with *Ceratostomella pini* in the present work indicate that this fungus is capable of causing a blockage of the water-conducting passages in the sapwood through internal wounding, which causes formation of resin. The resin is released in the sapwood and thus plugs up the pits between tracheids cutting off conduction of water. Reduction in water content of the stem follows as a result of withdrawal of water by the transpiring crown and inability of the water to pass up through the infected stem from the roots.

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PROBLEMS IN THE DETERMINATION OF PHYSIOLOGIC RACES OF *USTILAGO AVENAE* AND *U. LEVIS*¹

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In breeding plants resistant to disease, it is necessary to know the response of the varieties in use or of possible use in the breeding work to not only one collection but to a representative sample of collections of the pathogen. Therefore, determination of physiologic specialization of pathogenic organisms often is of great practical value.

Investigations on the oat smuts during the past 15 years have demonstrated repeatedly the existence of physiologic races. Reed (7) and Sampson (18), working with material of like origin, showed that 2 races of *Ustilago avenae* (Pers.) Jens. and two of *U. levis* (Kell. and Sw.) Magn. could be distinguished by their pathogenicity on certain oat varieties. Reed (8, 9, 10, 11, 13) and Reed and Stanton (16, 17) have continued this work and have demonstrated the occurrence of many specialized races. Schattenberg (19) and Radulescu (6) in Europe have found specialization with *U. avenae*.

For practical purposes a physiologic race in the cereal smut fungi can be defined as chlamydospore material that produces characteristic and fairly consistent effects on certain varieties of hosts, as measured by the percentages of plants affected, and sometimes also by the degree of smutting, in replicated and successive series of inoculations. Segregation and recombination may occur within races as defined above; hence replication in time is essential in their determination. There are several additional reasons why absolute consistency in the behavior of physiologic races cannot be expected. Among these are the following: variability in the host variety; in field studies different environmental conditions at time of infection in different years; the state of the inoculum as affected by the variety or varieties from which it has been obtained.

It is, therefore, important to determine the limits of variability under conditions that must be used in the practical determination of races.

It is obvious that pathogenicity, as a criterion for taxonomic separation, is not an absolute quality but one that is variable and dependent on 3 interacting factors, host, pathogen, and environment. The host need not be a factor in permitting variation, since cereal varieties can be obtained with a high degree of uniformity in their reaction to smut. A collection of smut chlamydospores cannot normally be expected to consist of a single biotype, but rather several biotypes, which tend to infect certain cereal varieties and produce chlamydospores. Moreover, it has been shown that not only intra-

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specific hybridizations in the oat smuts are easy to make but interspecific crosses also (1, 4). Thus opportunity is available for variation through hybridity in each chlamydospore generation. The host pathogen complex is unquestionably a factor in furthering variability in the pathogenicity of a chlamydospore collection. By the screening action of a normally resistant oat variety, Black Mesdag, on a potentially variable collection of *Ustilago avenae*, it has been possible to build up the pathogenicity of the collection to a state where Black Mesdag is no longer resistant (20). Environment acts on both host and pathogen. It has been shown clearly that the amount of smut produced on a variety of oats is dependent on favorable conditions of temperature and moisture for infection during the early seedling stages (12, 15). It has been shown, too, that varieties differ in their susceptibility to smut attack at different temperatures and moistures (15). There are no data available to show that smut collections respond to the environment, but they probably do.

A program was set up in 1933 at Minnesota to test the pathogenicity of many different collections of the oat smuts on oat varieties.² This program has been carried on with certain changes since that time, the major changes being the introduction for test of some new highly resistant varieties and the dropping from the tests of some varieties very susceptible to almost all races. It was evident early in the work that the pathogenicity of physiologic races cannot be confined within narrow limits; hence attempts to separate physiologic races on the basis of minor differences in pathogenicity are impracticable. This was at least partly due to the limited number of varieties in use and to the fluctuations in the amount of smut produced from year to year because the tests were made in the field. Some collections and some varieties have been tested each year throughout the entire period and these have served to indicate the extent of the annual variation in the amount of smut produced by the collections. Attempts also have been made to determine the importance of the host variety in altering the pathogenicity of collections by the selection of certain biotypes. The importance of different environmental factors as a cause for the year to year variation in pathogenicity has not yet been investigated.

MATERIAL AND METHODS

During the years 1933-35 and 1936-39, 79 collections of the oat smuts were tested at University Farm. Most of them had been obtained in the Mississippi Basin from Texas to Minnesota. Five varieties of oats: Anthony C.I. 2143, Gopher C.I. 2027, Iogold C.I. 2329, Black Mesdag C.I. 1877, and Markton C.I. 2053 have been used each year as tester varieties, while, in addition, during the last 4 years, 1936-39, Rusota C.I. 2343, D.C. II-22-220 ((Minota × White Russian) × Black Mesdag) C.I. 2874, Nakota C.I. 2883, Bond C.I. 2733, Navarro C.I. 966, and Fulgrain C.I. 3253 were included.

² This work was carried on by M. B. Moore during 1933 and 1934 and by E. K. Vaughan in 1936 and 1937, with the writer in charge since that time.

All seed was surface-sterilized by soaking in 1 to 320 formaldehyde for 5 minutes, draining and allowing to stand for 5 hours, and then washing in running water for 2 hours. For inoculum, chlamydo-spores were taken from a susceptible variety, usually Anthony, but occasionally Gopher and Iogold, in the case of most of the collections; or from Black Mesdag in the case of those collections that were able to produce smut on that variety. The his-

TABLE 1.—Amount of smut produced on 6 varieties of oats by collections of *Ustilago levis*

Year and variety on which inoculum was produced	1933	Anth. ^a
	1934	Min.	Min.
	1936	X	X	Goph.	Anth.
	1937	Iog.	Goph.	B.M.	B.M.	B.M.	B.M.
	1938	Anth.	Anth.	B.M.	B.M.	B.M.	B.M.
Variety inoculated and year of test	Percentage smut produced by different collections						
	8	96	104 ^b	105 ^b	114	117 ^b	
	Minn.	Minn.	Okla.	Kans.	Okla.	Kans.	
Anthony	1933	49
	1934	77	59
	1936	68	84	86	49
	1937	79	80	67	31	39	19
	1938	57	59	56	43	35	37
	1939	68	76	60	43	24	38
Gopher	1933	1
	1934	50	24
	1936	46	47	38	1
	1937	50	69	49	0	11	24
	1938	33	21	31	0	0	0
	1939	20	12	8	0	0	0
Iogold	1933	3
	1934	7	6
	1936	51	42	41	trace
	1937	67	69	62	1	7	6
	1938	26	16	14	0	0	0
	1939	33	21	2	0	0	0
Rusota	1936	49	32	41	2
	1937	27	42	42	0	3	22
	1938	28	7	11	0	0	0
	1939	16	28	20	0	0	1
Black Mesdag	1933	0
	1934	0
	1936	2	0	26	23
	1937	0	0	9	26	53	12
	1938	0	0	10	11	5	9
	1939	0	0	17	36	37	30
D. C. II 22-220	1934	0	0
	1936	0	0	17	12
	1937	0	0	4	28	28	1
	1938	2	0	12	13	10	16
	1939	0	0	28	27

^a Anth. = Anthony, Min. = Minrus, Iog. = Iogold, Goph. = Gopher, B.M. = Black Mesdag, X = not known.

^b Collections 104, 105, and 117 were described initially as *U. avenae*, but by using inoculum from Black Mesdag, the collections consisted of *U. levis*.

tory of chlamydospore inoculum for the collections to be considered in the paper is given in tables 1 and 2. The seed was inoculated by the partial vacuum method described by Zade (22) and modified by Haarring (3),

TABLE 2.—Amount of smut produced on 6 varieties of oats by collections of *Ustilago avenae*

Year and variety on which inoculum was produced	1933	X ^a	X	X
	1934	Min.	L.H.	J.-L.H.	Anth.
Year and variety on which inoculum was produced	1936	Anth.	Anth.	Anth.	Anth.	Goph.
	1937	Anth.	Anth.	Goph.	Anth.
	1938	Anth.	Anth.	Anth.	Anth.
Variety inoculated and year of test	Percentage smut produced by different collections						
	11	15	27	91	102	115	
	Texas	Okla.	Nebr.	Minn.	Okla.	Missouri	
Anthony	1933	21	36	30
	1934	70	73	59	59
	1936	80	85	54	79	30
	1937	89	76	49	77	13	85
	1938	37	95	31	51
	1939	34	56	25	53
Gopher	1933	trace	trace	trace
	1934	8	38	1	32
	1936	36	35	14	57	16
	1937	52	53	4	63	33
	1938	2	45	6	4
	1939	1	26	3	3
Iogold	1933	trace	1	0
	1934	4	3	1	13
	1936	21	42	4	83	4
	1937	69	70	1	78	7
	1938	3	54	2	11
	1939	2	36	0	8
Rusota	1936	43	57	34	60	19
	1937	55	73	16	72	26	60
	1938	15	60	8	12
	1939	11	22	17	19
Black Mesdag	1933	trace	0	0
	1934	0	0	0
	1936	1	trace	0	1	trace
	1937	0	1	1	1	0	2
	1938	0	0	0	0
	1939	0	0	0	0
D.C. II 22-220	1934	0	0	0	0
	1936	0	0	0	0	0
	1937	0	0	0	0	0	4
	1938	0	0	0	0
	1939	0	0	0

^a Anth. = Anthony, Min. = Minrus, L.H. = Liberty Hulless, J. = Joannette, X = unknown, Goph. = Gopher.

using a suspension of 0.5 g. of chlamydospores in 100 cc. water. The tests were made in duplicate by planting the seed directly in the field at the rate of five g. to an 8-ft. row. All heads of smutted and healthy plants were counted, the percentage of smut being based on heads rather than on plants.

EXPERIMENTAL RESULTS

Stability of Collections

Several of the collections have been tested for 4 to 6 years on the differential varieties and, although there is some variation in the amount of smut produced on one variety by the same collection from year to year, this variation is not sufficient to prevent recognition of races. Two collections of *Ustilago levis*, No. 8 from Minnesota and No. 105 from Kansas, have been consistently different in the 6 years they were tested (Table 1). Collection 8 has produced from 49 to 79 per cent smut on Anthony, 1 to 50 on Gopher, 16 to 49 on Rusota, 3 to 67 on Iogold, and 0 to 2 on Black Mesdag and D.C. II-22-220 (Fig. 1). On Gopher and Iogold, however, this collection has

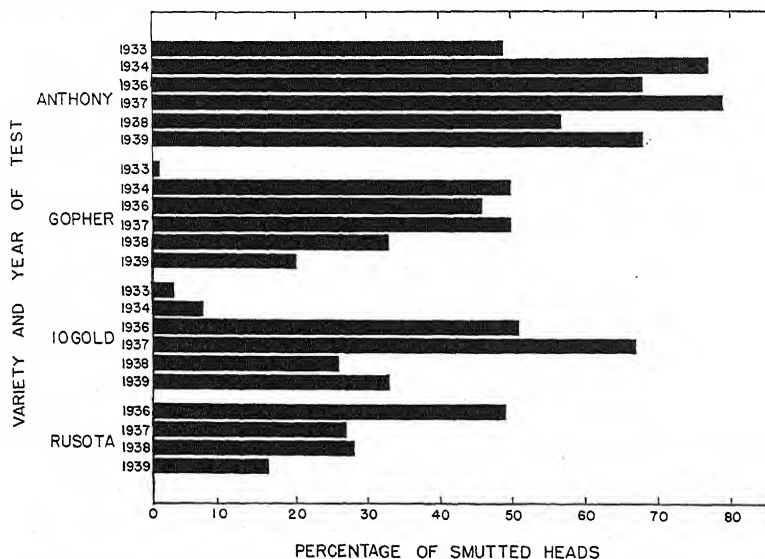


FIG. 1. Amount of smut produced by *Ustilago levis* No. 8 on 4 varieties of oats.

not been continuously uniform, for in 1933 there was only 1 per cent smut on Gopher as compared with 50 per cent in 1934 and never less than 20 per cent in succeeding years. Similarly, on Iogold, there was in 1933 only 3 per cent smut; in 1934, 7 per cent; and never less than 26 per cent subsequently. Obviously the pathogenicity of this collection changed during 1933 and 1934, possibly by the increased proportion of biotypes capable of attacking Gopher and Iogold, and then reached a state of equilibrium. With such a collection, the data of a single year are obviously insufficient to appreciate the potential pathogenicity of the smut. Collection 105, on the other hand, produced from 31 to 49 on Anthony, on Gopher 0 to 1, on Rusota 0 to 2, on Iogold trace to 1, on Black Mesdag 11 to 36, and on D.C. II-22-220, 12 to 28 per cent (Fig. 2). Similarly, collections 27 and 91 of *U. avenae* have been relatively consistent (Table 2).

The pathogenicity of some of the collections on the basis of the percentage of smut has shown rather wide variations which are not always easy

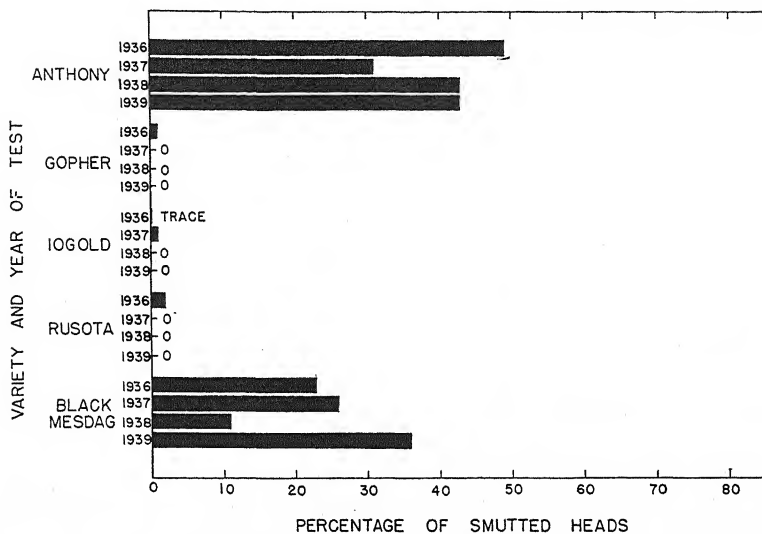


FIG. 2. Amount of smut produced by *Ustilago levis* No. 105 on 5 varieties of oats.

to explain. Variation may be due to environmental factors at seeding time and to the selective action of the previous host variety in tending to favor the production of biotypes to which it is most susceptible. Of the variable collections, one, No. 117 from Kansas, has been fairly constant on Anthony, 19 to 38 per cent smut, but, on Gopher, it caused 24 per cent smut in its first year of testing and no smut in the next 2 years. Similarly, in Rusota and Iogold, 22 and 6 per cent smut were produced, respectively, on these two varieties in the first year (1937) and none in the succeeding years. Black Mesdag had 12 per cent smut in 1937, 9 per cent the second year, and 30 per cent the third, while D.C. II-22-220 had 1, 16, and 20 per cent, respectively, in the 3 years (1937 to 1939) it had been tested (Fig. 3).

It is probable in the cases of Gopher, Rusota, and Iogold that the host, in this case Black Mesdag, on which the smut inoculum was grown for the

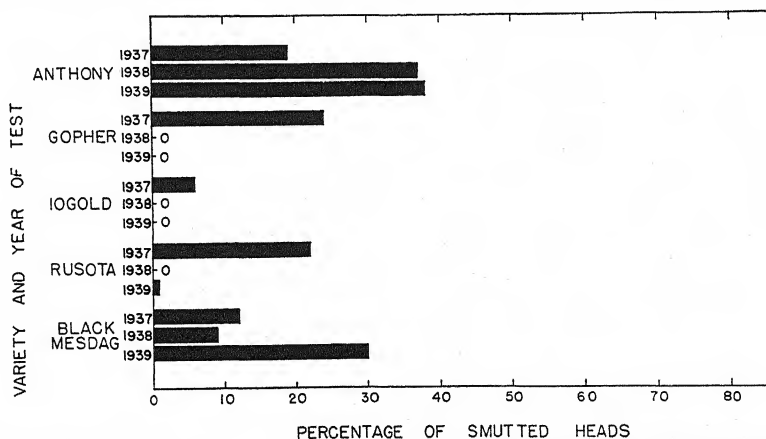


FIG. 3. Amount of smut produced by *Ustilago levis* No. 117 on 5 varieties of oats.

second and third year tests, exerted a selective influence in weeding out those chlamydospores that were capable of attacking Gopher, Rusota, and Iogold. But the variation in amount of smut on Black Mesdag could hardly have been due to any screening effect and more likely was due to unfavorable conditions for infection or unfavorable environmental conditions for the development of smut chlamydospores in the host.

The amount of infection caused by two collections of *Ustilago avenae*, 103 from Oklahoma and 109 from Kansas, also varied continually from year to year. Collection 103 in 1936 and 1937 caused moderate to heavy infection on Anthony, Gopher, Rusota, and Iogold. On Black Mesdag 30 per cent and 15 per cent smut was produced, respectively, in these 2 years and in D.C. II-22-220, 2 to 3 per cent infection. However, in 1937 and 1938 only Anthony and Black Mesdag were attacked and then only slightly. This loss of virulence on the part of the smut collection may be partly due to the screening action of the previous host variety. Table 3 summarizes the data obtained with collections 103 when the chlamydospores for inoculation were obtained from different oat varieties.

TABLE 3.—Amount of smut produced by selections of 2 collections of *Ustilago avenae*

Year and variety on which inoculum was produced	1936 1937 1938	Anth. ^a	B.M.	Anth.	Anth.	Anth.	Anth.	Anth.
		B.M.	B.M.	Anth.-Iog.	Ful.	Ful.	Bond	Bond
Variety inoculated and year of test		Percentage smut produced by different collections						
		103 Oklahoma			109 Texas			
Anthony	1936	65	22
	1937	90	87	15
	1938	9	56	17	11	15
	1939	24	3	1	2	3	2	trace
Black Mesdag	1936	30	trace
	1937	15	84	0
	1938	0	30	0	0	0
	1939	0	6	0	0	0	0	0
D.C. II-20-220	1936	2	0
	1937	3	76	0
	1938	0	33	0	0	0
	1939	0	0	0	0	0	0	0
Bond	1937	0	7
	1938	0	0	1	0	5
	1939	0	0	0	0	5	2	0
Fulgrain	1937	0	4
	1938	0	0	0	6	0
	1939	0	0	0	0	2	0	0

^a Anth. = Anthony, B.M. = Black Mesdag, Iog. = Iogold, Rus. = Rusota, Ful. = Fulgrain.

Chlamydospores of collection 103 in the first 2 years of test were taken from an unknown variety and from Anthony, respectively, and used to inoculate the tester varieties. In both 1936 and 1937 Anthony, Gopher,

Rusota, Iogold, and Black Mesdag were moderately to highly susceptible, while D.C. II-22-220 had only 2 and 3 per cent smut, respectively. But in 1938, with inoculum taken from Black Mesdag, only Anthony had any smut and then only 9 per cent. In 1939, smut taken from Anthony gave 24 per cent on Anthony and 2, 3, and 2 per cent, respectively, on Gopher, Rusota, and Iogold.

A selection of collection 103, obtained in 1936 by growing the smut on the resistant Black Mesdag variety gave, in 1937, 87 per cent smut on Anthony, 84 per cent on Black Mesdag, and 76 per cent on D.C. II-22-220. In 1938, this collection, again obtained from Black Mesdag, produced 56 per cent smut on Anthony, 30 per cent smut on Black Mesdag, and 33 per cent on D.C. II-22-220, while in 1939 there was a sudden drop in pathogenicity, with only 3 per cent on Anthony, 6 per cent on Black Mesdag, and no smut on D.C. II-22-220.

A comparison of these 2 selections from collection 103 indicates that the selection that in 1936, 1937, and 1938 had been increased on Anthony, Black Mesdag, and Anthony, respectively, decreased in virulence in 1938, not only in Black Mesdag but also on the very susceptible Anthony variety. However, the second selection, which had been increased only on Black Mesdag in the years 1936 to 1938, did not suffer a loss of virulence until 1939, a year later than the first selection. Also it differed in that Black Mesdag was still attacked, although only to the extent of 6 per cent.

It is very clear that in 1937, as Vaughan (20) has already reported, the screening effect of Black Mesdag increased the number of biotypes of the smut which could attack that variety. But it is not possible to explain as yet the subsequent loss of pathogenicity evinced by these collections in successive years.

Similarly, collection 109 has lost its power to produce smut on all of the tester varieties. This collection has always been weak in pathogenicity, for the amount of smut produced on Anthony in 1936 was only 22 per cent; in 1937, 15 per cent; in 1938, 7 per cent; and 1939, 1 per cent.

PHYSIOLOGIC SPECIALIZATION

Only 5 of the varieties of oats used to determine the pathogenicity of the oat smuts in the last 4 years are of value in the separation of physiologic races, namely, Anthony, Gopher, Rusota, Iogold, and Black Mesdag. Anthony is susceptible to all collections of *Ustilago levis* and *U. avenae* tested, while Black Mesdag and D.C. II-22-220 are moderately to highly resistant to a few collections and immune from the remainder. Gopher, Rusota, and Iogold are moderately to highly susceptible to most collections, although highly resistant to a few. Rusota is susceptible to more collections than either Gopher or Iogold. Because of the similarity in resistance of Black Mesdag and D.C. II-22-220 to all of the collections tried, it is only necessary to use one of these varieties as a differential host in distinguishing races. The remaining varieties in use, Markton, Nakota, Bond, Navarro, and Fulgrain have always been highly resistant to immune.

From tables 1 and 2, which compare the pathogenicity of several collections of *Ustilago levis* and *U. avenae*, respectively, on 6 oat varieties for several years, it is possible to separate distinct races of the oat smuts. In *Ustilago levis* there are 2 very distinct races with a third race, which is rather variable in reaction and acts like a combination of the other two. Race 1 is characterized by the susceptibility of Anthony, Gopher, Rusota, and Iogold and by the high resistance or virtual immunity of Black Mesdag (Table 1, collections 8 and 96). Race 2 (collections 105, 114, and 117) attacks Anthony severely and Black Mesdag moderately to slightly, with the remaining differential varieties, Gopher, Rusota, and Iogold, being highly resistant to immune. With collection 104, which serves as an example of race 3, the five varieties are all susceptible, although the percentage of smut produced on them, with the exception of Anthony, is very variable in the different years. It is well to note that collections 114 and 117 were able in the first year (1937) of their testing to attack Gopher, Rusota, and Iogold, while in the next 2 years these varieties were immune. Since the smut used for inoculum in 1938 and 1939 was obtained from Black Mesdag, it is likely that this variety had screened out the biotypes that possessed the ability to attack Gopher, Rusota, and Iogold. However, collection 104, despite its increase on Black Mesdag in 2 of the 3 years it was tested, always was able to attack, at least moderately, either Gopher, Rusota, or Iogold. It is, however, possible that continued increase of collection 104 on Black Mesdag may eventually select out biotypes that resemble race 2. This might be indicated by the progressive loss of ability to attack Gopher and Iogold, for, in 1937, Gopher had 49 per cent smut and Iogold 62 per cent, while, in 1939, it had but 8 per cent and Iogold 2 (Fig. 4). On the other hand, Rusota, although being less heavily attacked in 1938 and 1939, did not show a progressive

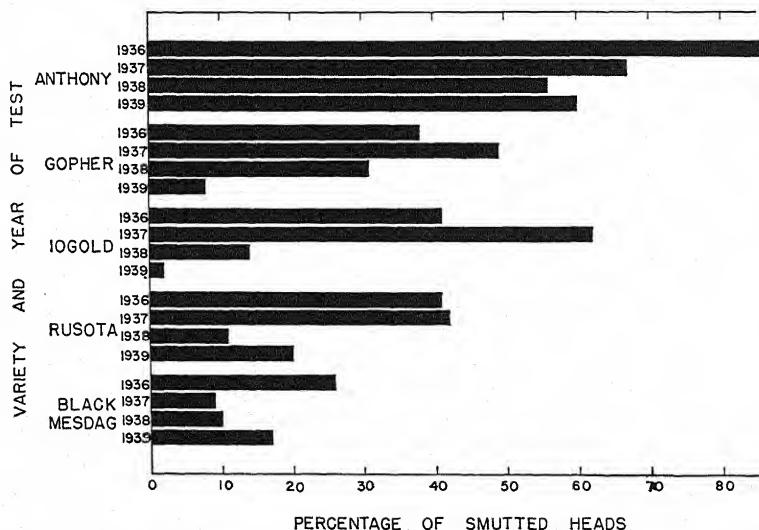


FIG. 4. Amount of smut produced by *Ustilago levis* No. 104 on 5 varieties of oats.

decrease in the amount of smut, for the percentage in 1939 was 20 after the low amount of 11 per cent in 1938. At the present moment with collection 104 it is difficult to say whether it comprises a relatively permanent race, is a mixture of two or more races, or whether its fluctuation in pathogenicity on Gopher and Iogold is due to environmental changes. If it consists of more than 1 race, the use of selective varieties might allow the segregation of races, clearly separable on the basis of pathogenicity.

In *Ustilago avenae*, 3 races can be distinguished (Table 2). With race 1 (collections 27, 115, and possibly 102) only Anthony and Rusota are susceptible, although Gopher and Iogold usually are attacked to a slight degree. Black Mesdag is immune. Race 2 (collections 11, 15, 91) is distinguished by the susceptibility of Anthony, Gopher, Rusota, and Iogold and the resistance of Black Mesdag. This race comprises most collections of *Ustilago avenae* tested at Minnesota. The type of race 3 is collection 103, mentioned in table 3. Collection 103 in 1936, the year it was first observed, attacked all 5 differentials with moderate severity. In 1937, when increased on Anthony, it behaved much as in 1936, except that Black Mesdag was less severely attacked. When inoculum of this race was obtained from Black Mesdag and grown in 1937 on Anthony, Gopher, Black Mesdag, and D.C. II-22-220, it was found that the amount of smut produced on Black Mesdag had risen from 15 per cent, using inoculum from Anthony, to 84 per cent, when the inoculum came from Black Mesdag (Table 3). Similar results were obtained in the case of D.C. II-22-220. It is unfortunate that in succeeding years it was not possible, for reasons yet undetermined, to maintain the pathogenicity of this smut.

SCREENING TESTS

From 1936 onwards, attempts have been made to select certain biotypes from the general collections. Vaughan (20) already has reported the selection of 2 races of *Ustilago avenae* that attack Black Mesdag. Both of these races have been discussed above, Vaughan's Oklahoma race being designated as collection 103, and the Kansas race as collection 105. Collection 103 has since become less pathogenic and no longer resembles the collections tested in 1936 and 1937. Collection 105—the Kansas race—is, however, not *U. avenae* but *U. levis*. It is possible that in 1936, collection 105 consisted of a mixture of *U. levis* and *U. avenae* or else a hybrid of these species. By using for inoculum smut from Black Mesdag, only *U. levis* was perpetuated.

Similarly, collection 117 in 1937 was identified as *Ustilago avenae*. In that year the 5 oat varieties, Anthony, Gopher, Rusota, Iogold, and Black Mesdag, were attacked. By using smut from Black Mesdag as inoculum in 1938, a collection of pure *U. levis* was obtained to which only Anthony and Black Mesdag were susceptible but not Gopher, Rusota, or Iogold (Fig. 3 and Table 1).

Because collection 105, Vaughan's Kansas collection, is not *Ustilago avenae* but *U. levis* and because collection 103, Vaughan's Oklahoma collec-

tion, has become incapable of producing more than a small amount of smut on any of the differential varieties, no collections of *Ustilago avenae* capable of attacking Black Mesdag are at present available.

In some years some of the varieties normally immune from the oat smuts here, may have up to 5 per cent smut. It is, of course, possible in these cases that the varieties in use may have a slight admixture of a susceptible variety, but in certain cases plants are attacked that correspond unquestionably to known immune varieties. In 1937, Bond and Fulgrain were attacked by a collection of loose smut (collection 109) to the extent of 7 and 4 per cent, respectively (Table 3). Inoculum for 1938 was taken from Bond, Fulgrain, and a mixture of Anthony and Iogold, and these varietal selections were run separately on Anthony, Bond, and Fulgrain. The Anthony-Iogold selection caused 7 per cent smut on Anthony, 1 per cent on Bond, and produced no smut on Fulgrain. The Bond selection gave 15 per cent of smut on Anthony, 5 per cent on Bond, and none on Fulgrain; while the Fulgrain selection gave 11 per cent on Anthony, none on Bond, and 6 per cent on Fulgrain. Because of the weak pathogenicity of this collection at University Farm, it is not wise to attempt any general conclusions. It might be well, however, to state that in 1938 smut from Bond attacked Bond and smut from Fulgrain attacked Fulgrain, although the amount of smut produced was very small. In 1939, continuation of this screening test did not, however, give any further data because of the virtual failure of any selections of collection 109 to attack even the susceptible Anthony to an extent greater than 3 per cent. One of the selections did attack Bond to the extent of 5 per cent.

Only in Bond and Fulgrain of the resistant oat varieties has smut been produced at all consistently. The maximum amount ever developed on Bond has been 7 per cent and on Fulgrain 6 per cent. Navarro, Markton, and Nakota have had an occasional smutted plant, but in these cases the infected plant may have been off-type.

Thus attempts to alter the pathogenicity of collections by taking for inoculum chlamydospores from resistant varieties has been, in general, unsuccessful. As indicated above, only with a few collections has it been possible to increase the amount of smut produced on Black Mesdag and D.C. II-22-220 by using for inoculum chlamydospores from the former variety. No success has been obtained in using chlamydospores from Bond and Fulgrain to reinoculate the varieties that produced the inoculum. Bever (2) recently reported a similar lack of success with purified physiologic races of *Tilletia tritici* and *T. levis*.

DISCUSSION

In conducting studies in physiologic specialization of the smuts, there are several factors that tend to make for variation in the data from year to year. Field experiments, of course, do not permit the maintenance of optimum conditions for the development of smut and the differentiation of collections

on the basis of pathogenicity. The index of pathogenicity used in determining the pathogenic reaction of different smut collections is chlamydospore production. Western (21) has pointed out that penetration of the host tissue by smut mycelium does not mean that chlamydospores will eventually be produced, for, smut mycelium in uncongenial varieties may persist for some time, although no external signs of its presence may be observed. It is highly probable, too, that with the cereal smuts, varieties may, also, under certain environmental conditions, be truly infected but carry a dormant mycelium. Under field conditions where little or no control of important environmental factors is feasible, great variation in the total amount of smut produced on any one variety from year to year is possible.

Some of the factors that are of importance in making for variation in smut production are as follows:

The Environment at Time of Seeding. The optimum conditions for infection in the oat smuts are a soil temperature of about 25° C. and a low soil moisture (15). At temperatures and soil-moisture contents varying from these optima, a lessened production of smutted heads would be expected. It is, however, possible that different collections of smut may have different optimum conditions for infection or that there may be differences in varietal resistance under different environments. Reed and Faris (15) have shown that low soil temperatures (5° C.) at seeding render *Avena nuda* var. *inermis* more susceptible than *A. sativa* var. *Victor* to *Ustilago levis*. On the other hand, at high temperatures (30° C.), Victor is more severely attacked. Also the moisture content of the soil at the time of infection is important. It might be expected also that smut collections differ in their response to temperature and soil moisture. Consequently, variation in the amount of smut produced from year to year would arise from, among other possible factors, the action of temperature and soil moisture on (a) the host variety (b) the smut collection, and (c) on the future development of the smut mycelium within the host.

Post-infection Environment. It is possible that unfavorable environment, after infection has taken place, may lead to variation in smut production. Reed (12) states that the factors of the environment most effective are those that act during very early seedling stages; but Nicolaisen (5) found that too high or too low light intensity inhibited spore formation in *Ustilago avenae*, under greenhouse conditions. Reed (14), however, states that there does not appear to be a definite relation between growth of the host and the ultimate expression of smut.

Selective Action of the Host Variety. The host variety on which a collection of smut is grown tends to perpetuate those biotypes to which it is most susceptible. In this way it is possible to modify the pathogenic response of smut collections. Because of this screening effect, the source of inoculum from year to year may have an important result. This has been noted earlier in the discussion of collection 117, which at one time consisted of a mixture of *Ustilago levis* and *U. avenae* and from which it was possible

to select, by using for inoculum smut from one variety—Black Mesdag—a race of *U. levis* differing in pathogenicity from the mixed collection. It is doubtful whether attempts should be made to perpetuate collections in a relatively uniform state from year to year, by including all biotypes from all varieties attacked or whether the inoculum should be taken from selected varieties. Even with the first method it would not be too certain that the collection would remain constant from year to year, while the second method unquestionably would lead with certain varieties and collections to the establishment of a race differing from the general collection. Practically, it is important to determine the resistance of all varieties under test, especially those serving as parental stock in the production of disease-resistant varieties. With this in mind, it is best to take what advantage one can of the screening action of varieties and to attempt to determine the widest limits of pathogenicity of one collection by trying to increase in number those biotypes attacking important parental varieties.

SUMMARY

Eleven varieties of oats were inoculated with 79 collections of the oat smuts, 17 of *Ustilago levis* and 63 of *U. avenae*, collected principally in the Mississippi Valley basin.

Some of the collections were not constant in their ability to attack the same variety in succeeding years, taking one to two years to become stable in their reaction.

In some cases this variation is known to be due to the action of the host variety, on which the chlamydospores for inoculum were grown, in exercising a selective effect on the collection by increasing certain biotypes of the smut at the expense of others.

It is likely that, in the field studies, the varying environment from year to year must play a part in making for variation in the pathogenicity of any collection.

Three races of *Ustilago levis* and 3 of *U. avenae* can be distinguished on the basis of their pathogenicity on 5 oat varieties, Anthony, Gopher, Rusota, Iogold, and Black Mesdag.

Attempts to build up races of *Ustilago avenae*, which attack slightly Bond and Fulgrain, by repeated increase of them on these two varieties have been unsuccessful.

Markton, Navarro, and Nakota have not been attacked by any collection, while D.C. II-22-220 resembles Black Mesdag closely in its reaction to smut.

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SOIL ROT OF SWEET POTATOES IN LOUISIANA

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Following a very serious outbreak of the soil rot or pox of sweet potato in the Sunset region of Louisiana, the most important sweet-potato area in the State, a study was made of the disease. In this paper is presented the information obtained in regard to the symptoms of the disease and its cause. The investigations on control measures, especially on the value of soil treatment with sulphur, will be included in a later paper.

The first published mention of this disease was apparently made by Halsted (3) in 1890. He applied the name "soil rot" to it and stated that it was caused by the fungus *Acrocystis batatas* E. and H. In 1916, Elliott (2) published the results of an extensive study in which he claimed that the disease was caused by *Cystospora batata* Ell. In 1918, Taubenhaus (11),

while agreeing with the results of Elliott, mentioned that an actinomycete, to which he gave the name *Actinomyces poolensis* Taub., was associated with the pox lesions. He stated, however, that "*Actinomyces poolensis* is a superficial wound parasite usually found following the pox spots produced by *Cystospora batatas*." Manns (5), after removing the covers from slides of pox material that had been stained by Elliott and restaining them with Ziehl-Neelsen's carbol-fuchsin, found an actinomycete present in every case. Preliminary work by Manns had indicated that the disease was induced by an actinomycete.

Further studies by Manns and Adams (6, 7, 8, and 9) proved definitely that pox or soil rot of the sweet potato was caused by an actinomycete to which they applied the name *Actinomyces Pox*.

In 1929 Adams (1), reporting on the cause of soil rot, referred to the organism as *Actinomyces p.* He compared a culture of *A. poolensis*, which he had obtained from S. A. Waksman, with cultures of *A. p.* and believed them to be different. He found *A. poolensis* to be nonpathogenic, whereas *A. p.* produced typical pox lesions.

DISEASE SYMPTOMS OBSERVED IN LOUISIANA

Two rather distinct types of symptoms are associated with soil rot or the soil-rot complex of diseases in Louisiana. (1) A disease characterized by more or less round, sunken, black to brownish lesions in which the epidermal

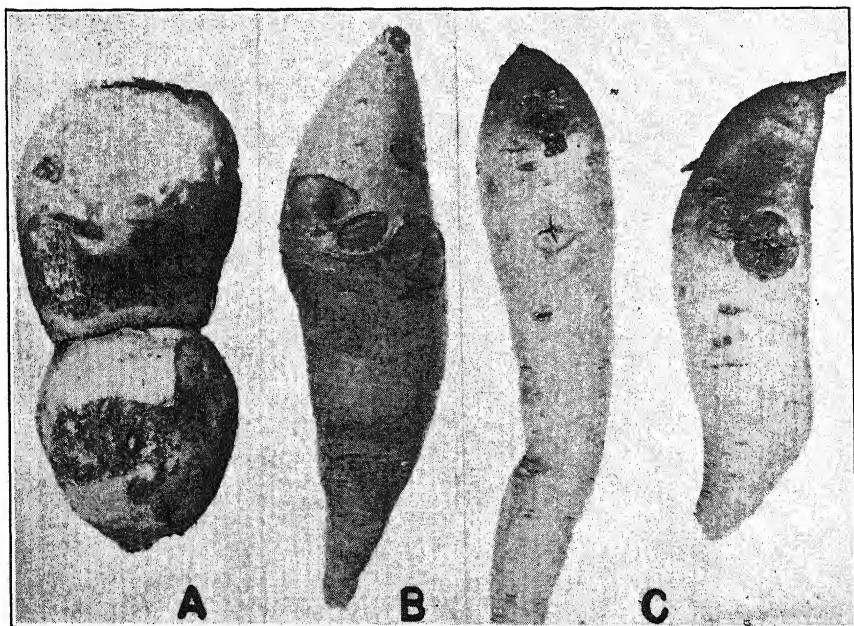


FIG. 1. Types of lesions found on sweet potatoes. A. Potato with typical lesions of soil rot, from naturally infested soil. B. Appearance of soil rot after the invaded tissue had sloughed off. C. Lesions, with the appearance of those described by Halsted in 1890.

layers have a tendency to split and the lower layers to disintegrate and become more or less friable upon drying (Fig. 1, C), has been known in this State since 1922. The symptoms are apparently identical to those first illustrated by Halsted (3). Potatoes bearing such lesions have been seen from time to time in various parts of the State, but only rarely has the injury been sufficient to cause any concern, though, in 1939, several lots of potatoes were rejected for shipment in St. Landry Parish because of severity of the infection. As lesions typical of those of this type occurring in the field have not been produced by artificial inoculation, the place that the disease with these symptoms has in the soil-rot complex is not clear. (2) The disease now known locally in Louisiana as soil rot, the one to which the term soil rot definitely applies in the following pages of this paper, has symptoms quite unlike those mentioned in the preceding paragraph.

The symptoms of soil rot as observed in the field differ from those of any other sweet-potato disease. In heavily infested soil the plants are dwarfed and make little or no vine growth (Fig. 2). The plants appear as though conditions were unfavorable for growth, the leaves being small and pale green to yellow. Many of the plants die before the end of the season. The diseased plants are easily pulled from the ground and the root system is very poorly developed, most of the roots being entirely rotten and many of them breaking off when the plant is lifted from the soil (Fig. 3). Small, elongated, dark-colored lesions may also be present on the stem below the soil line. The disease moreover is present on the mature potatoes in the

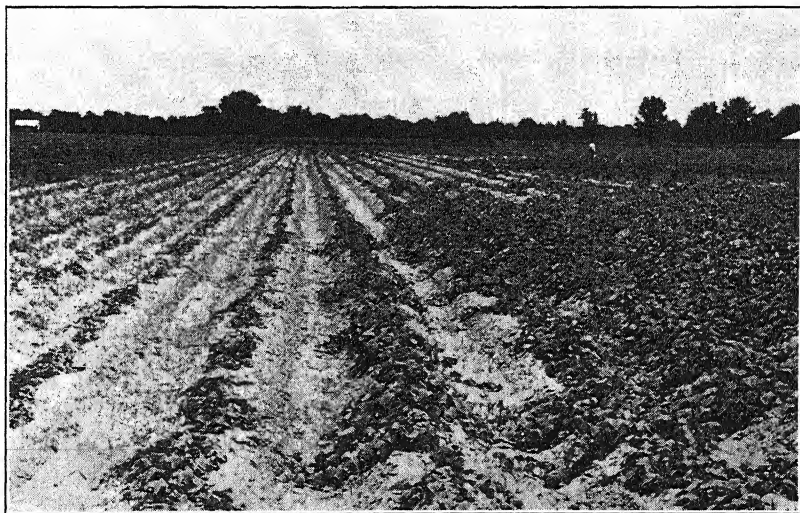


FIG. 2. Sweet-potato field at Sunset, Louisiana. The soil rot had prevented growth of plants on the left and was spreading into the remainder of the field.

form of pits or cavities with irregular jagged or roughened margins (Fig. 1, A). The lesions may vary from one-quarter inch to more than an inch in diameter, sometimes coalescing and covering most of the surface of the



FIG. 3. Plants from an infested field. Plants badly stunted and with but few roots.

potato. In the early stages of the disease the lesions are covered by the skin of the root, but this later breaks away, exposing the pits. These are slightly sunken and the new epidermal layer, apparently perfectly normal, is covered with the black, granular remnants of the old, dead tissue (Fig. 1, B). A potato, infected while young, may be entirely girdled and as it continues to grow, may enlarge on each side of the infection center and thus become badly misshapen.

OCCURRENCE AND IMPORTANCE OF SOIL ROT

The soil rot characterized by symptoms described in the preceding paragraph was first observed and recognized in Louisiana in 1934. In that year a few small spots in fields near Sunset, St. Landry Parish, showed the disease in very severe form. In subsequent years it spread rapidly and is now known to be present to a greater or less extent in many of the commercial growing areas in the State. In St. Landry Parish, where we have the largest production of sweet potatoes in the United States, conditions seem particularly favorable for the development of the soil rot. The disease seems now to be the most important limiting factor. Numerous fields in which the crop was totally destroyed have been observed and a number of farms have become so thoroughly infested that sweet-potato production has been aban-

doned. Losses in Louisiana agree well with those reported from Texas, New Jersey, Maryland, Delaware, and Virginia, where the disease has been severe.

ENVIRONMENTAL RELATIONSHIPS

The severity of soil rot is modified very considerably by two environmental factors, the water content and hydrogen-ion concentration of the soil.

A high or satisfactory water content of the soil stimulates a more rapid development of roots and also makes it possible for a diseased plant with a very deficient root system to absorb more easily the water and essential mineral salts from the soil. In a rainy season, sweet potato plants affected with soil rot are able to make vines and often fairly satisfactory yields, but this is not the case in seasons of deficient rainfall. In the wet season of 1939, certain fields in the vicinity of Sunset, known to be severely infested with the soil rot organism, produced fairly satisfactory yields, though the potatoes showed a high percentage of lesions. In the 3 or 4 preceding years, which were considerably drier, the production in these fields was very unsatisfactory.

From investigations in Louisiana, soil rot does not develop in soils of pH 5.2 or below. Most soils in the Sunset area show a pH of 5.8 to 6.2. In such soils, the disease apparently develops most satisfactorily. It has been possible practically to eliminate it by adding sufficient sulphur to lower the pH to 5.0.

ISOLATION OF CAUSAL ORGANISM

In the early attempts to isolate the organism responsible for the disease, Taylor's method (12) for isolating *Actinomyces scabies* was used. Later, another method, described below, was adopted and gave somewhat better results.

A lesion and a small portion of the underlying healthy tissue are removed from an infected root and placed in a 3 per cent solution of calcium hypochlorite for 2 minutes. The disinfected lesion is then removed, and, without washing, is macerated in a tube of melted Ashby's Mannitol agar. The agar is poured into a sterile Petri dish and incubated at 32° C. for 3 to 7 days. The actinomycete colonies that develop in the plates originate mostly from the small macerated pieces of the diseased tissue.

Numerous isolations have been made from soil-rot lesions on the roots, mature potatoes, and underground portions of the stems. From most of the platings actinomycete colonies have appeared within 3 to 7 days, and from transfers to tubes of Difco dextrose agar several types of actinomyces have been obtained. However, from young infection spots there has appeared a predominating type of actinomycete, capable of producing soil rot symptoms when used in inoculation tests.

PATHOGENICITY

In order to test the large number of isolates obtained, a quick laboratory method was devised which has given very good results. Succulent sweet-

potato stems were cut into portions containing 1 or 2 nodes, sterilized in 1:1000 mercuric chloride solution for 2 minutes, washed with sterile water, and placed on water agar in large, 150 mm. Petri dishes. The dishes were kept in the dark at room temperature for 4 days during which time rootlets developed at the nodes. The rooted cuttings were then transferred to sterile water-agar dishes, and small agar blocks, on which were actively growing cultures of the organisms to be tested, were inverted on the young roots. The inoculated rootlets were kept at 32° C. for 4 to 6 days. In this period of time a parasitic isolate usually produced a rot of the rootlet immediately below the agar disk (Fig. 4). The root tissues were completely broken down, resembling very closely the type of infection found on young rootlets under natural field conditions. There was little progressive rot of the rootlet beyond the point of inoculation. By this method a large number of isolates were tested. While this test in itself was not sufficient to prove definitely the pathogenicity of the various isolates, it was sufficiently accurate to eliminate the nonpathogenic ones.

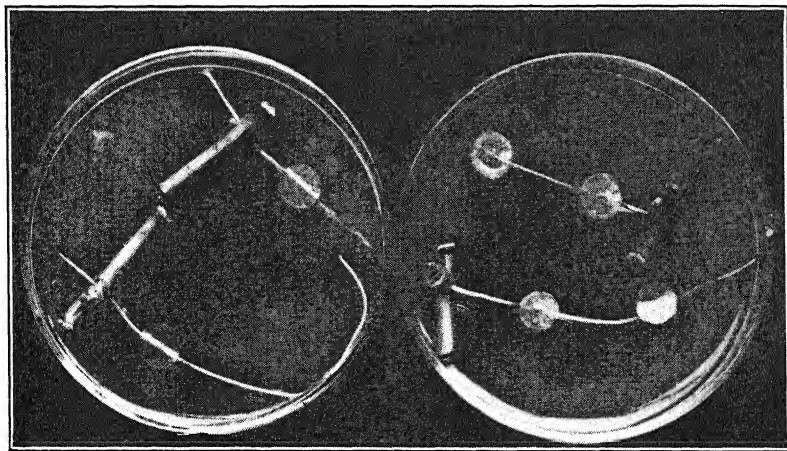


FIG. 4. Petri-dish method for testing the pathogenicity of *Actinomyces* isolates.

Isolates, found to be parasitic in the Petri-dish tests, were also inoculated into freshly-dug sweet potatoes. The inoculum was placed in slight punctures near each end of a potato. The potatoes were placed in moist chambers and incubated at 32° C. A number of these isolates in 5 days produced typical soil rot lesions at the point of inoculation.

A few cultures of *Actinomyces* were obtained that were slightly parasitic but distinct from those that produced definite soil-rot symptoms. As an example, one of these (Isolate G) produced a slightly raised blister-like lesion in which the tissue was slightly darkened, but not killed, as in typical soil-rot lesions. Small localized lesions also developed on the rootlets, but there was no noticeable stunting of the plants in either greenhouse or field tests.

A number of parasitic isolates that produced typical soil-rot lesions in laboratory tests were tested in the greenhouse with growing plants. The inoculum was increased on a mixture of equal parts of sifted horse manure and soil which had been sterilized for two hours. After the cultures had grown for 8 to 10 days, the inoculum was mixed into the soil in which sweet potato plants were to be grown. An equal amount of the sterilized manure-soil mixture was added to soil to be used for the controls. Two soil types were employed, a fine silt loam of the Olivier series, obtained from a field infested with the soil rot organism, and a heavy clay loam of the Sharkey series, in which potatoes had never been grown. The soil was sterilized for 2 hours on 2 successive days before the inoculum was added. The Puerto Rican variety was grown in all experiments.

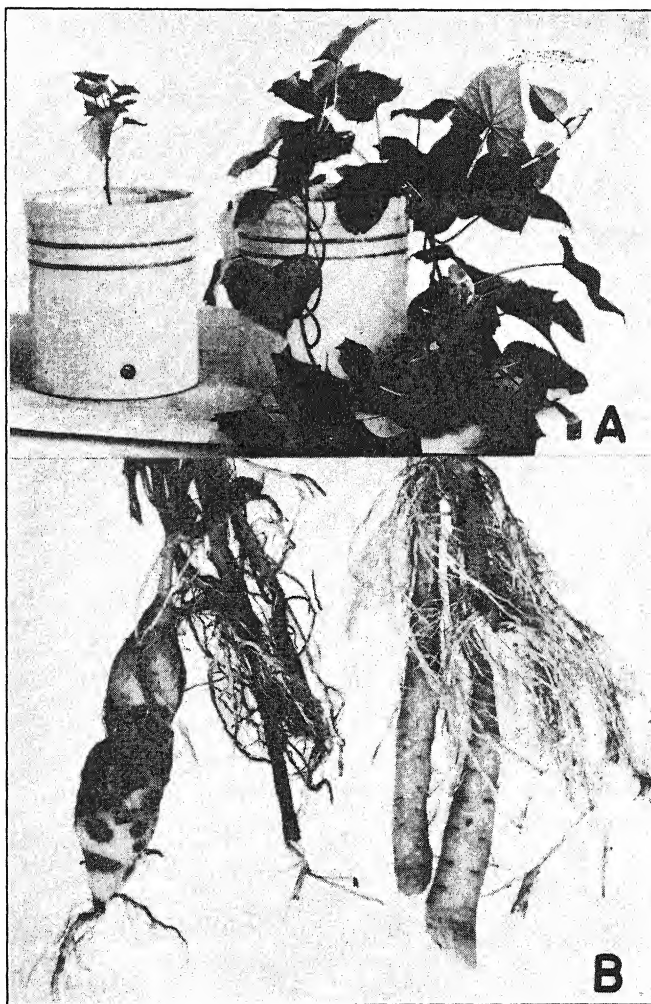


FIG. 5. Inoculation test with *Actinomyces ipomoea*. A. Plant (left) in soil inoculated with *A. ipomoea* compared with one (right) in sterilized soil. B. Roots from inoculated plant (left) compared with those from the sterilized soil (right).

In the first test 3 crocks of each soil type were inoculated with each isolate and 3 crocks were left as controls. Isolates of proved pathogenicity under laboratory test, and 2 similarly found to be nonpathogenic were included. Three potato cuttings were set in each crock. At the end of 3 and 5 weeks, respectively, a cutting was removed from each crock. The roots of the plants that were grown in soil inoculated with the pathogenic cultures showed typical symptoms of soil rot at the end of 3 and 5 weeks, while the roots of those removed from soil inoculated with the 2 nonpathogenic isolates were well developed, free from lesions, and indistinguishable from the control plants. At the end of 3 months when the experiment was terminated the plants in the soil inoculated with pathogenic isolates were small and stunted, while those in the noninoculated soil and in the soil inoculated with nonpathogenic isolates had grown and vined normally (Fig. 5, A). The stunted plants had poorly developed root systems, with very few secondary roots. Numerous soil-rot lesions were found on the larger roots and on the small potatoes (Fig. 5, B).

The plants from soil inoculated with the nonpathogenic isolates were as large and vigorous as those from the soil of the controls, and showed no evidence of soil-rot lesions on the roots. Similar results were obtained with each soil type.

In another test, inoculum of various isolates was mixed with sterilized soil in 6-in. pots 5 days before the cuttings were set. Five isolates were tested, 6 pots being used for each isolate. In addition, 6 pots of nonsterilized, naturally infested soil were included in this test. One cutting was set in each pot. The pots were kept in the greenhouse for 7 days, then removed to the field and buried in the soil with about 1 inch of each pot remaining above the soil surface. Typical stunting of the plants occurred in all of the inoculated pots, and these plants could not be distinguished from those growing in the naturally infested soil. The control plants in sterilized soil grew normally.

Inoculation tests also were conducted in the field. The inoculum used was a pure culture of the *Actinomyces* growing on a manure-soil medium. One pint of this inoculum was placed in the row in each spot where a sweet potato cutting was to be planted, and was mixed in approximately the upper 6 inches of soil. Five days after applying the inoculum the cuttings were set. A nonparasitic isolate (Isolate C) and a parasitic isolate (Isolate 35) were used in this experiment.

In the first set of field inoculations made on June 4, 15 hills were inoculated with each isolate and 15 hills were left as controls. In the second test 20 hills with each isolate were used. The plants in the hills inoculated with the parasitic isolate remained small and stunted (Fig. 6, A), while the control plants and those inoculated with the nonparasitic culture grew normally (Fig. 6, B). The yields obtained from the 2 sets of inoculations are shown in table 1.

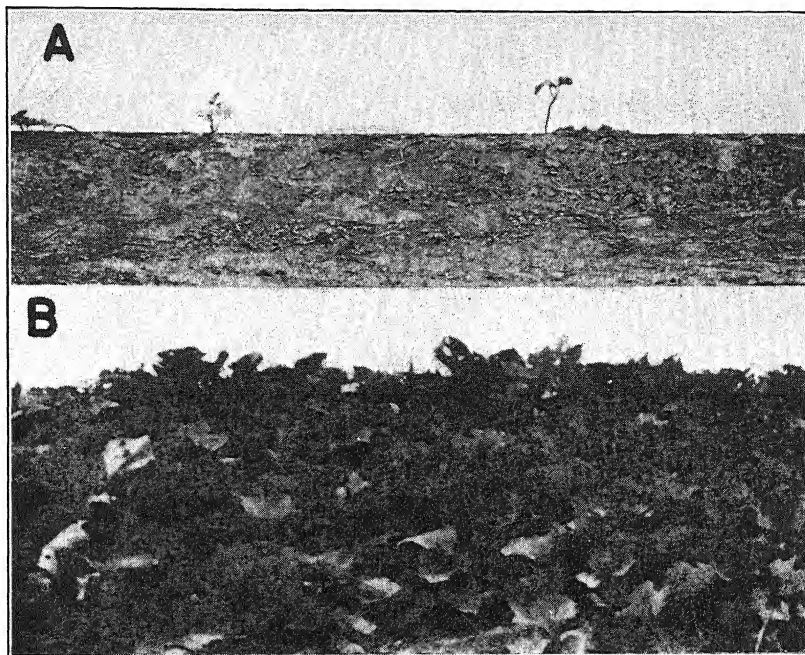


FIG. 6. Inoculation test in field with *A. ipomoea*. A. Plants in inoculated soil. B. Plants in noninoculated soil.

TABLE 1.—Yields of sweet potatoes obtained from field-inoculation experiments on a silty clay-loam soil

Isolate	Test 1 June 4 to October 13		Test 2 June 18 to November 9	
	Marketable lb.	Culls lb.	Marketable lb.	Culls lb.
35, parasitic	0.0	2.4	3.5	4.0
C, nonparasitic	10.9	7.9	15.0	3.5
Control	10.3	2.1	14.0	7.0

As shown in table 1, the actinomycete isolate that caused a stunting of the plant growth in the field also caused a marked reduction in yield of marketable potatoes.

A number of cross-inoculation tests have been made, but no infection has yet been obtained on Irish potatoes with the sweet potato isolates of *Actinomyces* nor on sweet potatoes with the Irish potato scab organism, *Actinomyces scabies*.

PHYSIOLOGICAL STUDIES

The relation of the soil-rot *Actinomyces* to temperature was determined by growing streak cultures of different isolates on Difco dextrose agar in incubators at temperatures ranging from 15 to 55° C. Three different isolates were used, and in the tests there were included 3 plates of each isolate.

The plates were examined after growing for 8 days in the incubators, and the amount of growth estimated. In table 2 are included the results of the various tests.

TABLE 2.—*The growth of the soil-rot Actinomyces on Difco dextrose agar at different temperatures*

Isolate	Temperature °C.							
	15	20	27	32	37	42	45	55
35	— ^a	—	++	+++	++	+	—	—
105	—	—	++	+++	++	+	—	—
160	—	+	++	+++	++	+	—	—

^a—No growth; + slight growth; ++ moderate growth; +++ abundant growth.

Isolate 160, obtained in northern Louisiana, made slight growth at 20° C., while isolates 35 and 105, obtained at Sunset, failed to grow at that temperature. The optimum temperature for all isolates was in the neighborhood of 32° C.

In the report on the soil-rot *Actinomyces* made by Adams (1), he stated that the optimum temperature for growth was between 30° and 37° C., and that there was very little growth at 52°. In the studies made in Louisiana there was no growth at 45° C. and very little at 42°.

The relation of the soil-rot *Actinomyces* to hydrogen-ion concentration was also determined. The 3 isolates used in the temperature studies were grown in glycerol-asparagin solution adjusted to different hydrogen-ion concentrations. Three tubes, each containing 10 cc. of the medium, were used for each isolate in each test. The cultures were examined after incubating for 8 days at 32° C. The results of the tests are included in table 3.

TABLE 3.—*The relation of hydrogen-ion concentration to the growth of the soil-rot Actinomyces*

Isolate	Hydrogen-ion concentration						
	4.6	4.8	5.0	5.2	5.4	5.6	7.0
35	—	—	—	++	++	+++	+++
105	—	—	—	++	++	+++	+++
160	—	—	+	++	++	+++	+++

Isolate 160 showed a very slight growth at pH of 5.0, while the minimum for the other two was 5.2. The best growth was observed at pH 5.6 to pH 7.0. The results obtained with cultures agree with those obtained in the field. When sulphur has been applied to the soil in sufficient amounts (600 to 800 lb. per acre) to reduce the pH of the soil to approximately 5.0, very effective control of the disease has been obtained.

DESCRIPTION OF ORGANISM

From the studies that have been made on the actinomycete causing soil rot of sweet potato in Louisiana, it is evident that it is an undescribed spe-

cies. In the past, species of *Actinomyces* have been associated with soil rot by Taubenhause (11) and by Manns and Adams (6, 7, 8, 9).

Taubenhause described *Actinomyces poolensis* as occurring on sweet potato, but stated that it was a superficial wound parasite and not the cause of soil rot. Adams (1) later studied a culture of *A. poolensis* and found that it was not parasitic on sweet potato. That the *Actinomyces* associated with soil rot in Louisiana is distinct from *A. poolensis* is confirmed by a report from S. A. Waksman. After studying a culture received from Louisiana, he reported as follows:

"This organism is quite distinct from the form isolated by Taubenhause and submitted to me some twenty years ago, which has been later described as *Act. poolensis*. Your organism represents a typical *Actinomyces* and is characterized by the formation of an aerial mycelium forming spiral-shaped chains of spores. The most characteristic aspect of this organism is the greyish-green to almost green color of the aerial mycelium on certain synthetic medium such as dextrose agar, as well as on potato dextrose agar. The organism is strongly proteolytic; it liquefies gelatin rapidly without the production of any pigments; it rapidly dissolves the casein in milk, changing the reaction to alkaline. It grows in glucose solution in the form of heavy flakes which settle to the bottom; it does not produce any surface growth on liquid media, at least those that we have tested. Growth on agar media is cream-colored, rough and folded; aerial mycelium is either absent as on nutrient agar or is present covering only certain areas; it is either white with occasional mouse-grey patches or is greyish-green to green in color. It does not produce any characteristic growth on potato slants, although it grows readily in the form of a cream-colored, folded mass.

"On the basis of these data, I feel that you are justified in describing this organism as a new species."

Manns and Adams (6, 7, 8, 9) and later Adams (1) studied an actinomycete, isolated from sweet potatoes, and referred to it as *Actinomyces Poz* and *A. p.* This organism was parasitic on sweet potatoes and produced definite soil-rot symptoms. It was very probably the same as the one being studied in Louisiana, but it was not described as a new species.

As the sweet-potato soil-rot actinomycete is apparently an undescribed species, it is here being described as *Actinomyces ipomoea*, sp. nov. The following description is based on a study of 4 different isolates. These were grown on culture media made up according to the formulae used by Waksman (13) in his study of *Actinomyces*. All tubes of media were inoculated as uniformly as possible using aerial mycelium and conidia. The tubes were incubated at 32° C. for 20 days, unless otherwise stated. Cultural characters are based on at least 2 and in some cases 3 tests.

Actinomyces ipomoea, sp. nov.

I. Morphology.

1. Spirals.

Partly spiral-shaped chains of oval to elliptical spores formed in aerial mycelium on dextrose-casein, egg-albumen, and dextrose agar.

2. Conidia.

Dextrose-casein agar: Oval to elliptical, 0.9 to 1.3×1.3 to 1.8μ .

II. Cultural Characteristics.

1. Synthetic agar—(saccharose).

Growth: Abundant, mostly on surface of medium, moderately wrinkled; color, close to olive yellow (10).

2. Dextrose agar.

Growth: Abundant, deep in medium, wrinkled; color, nearly new silver (4) with yellowish tint at base of slant.

Aerial mycelium: Moderate amount, mostly white, later shading to a bluish green.

Soluble pigment: None.

3. Nutrient agar.

Growth: Moderate, in the form of small, shiny, crinkled colonies both on the surface and imbedded in the medium; color, new silver (4).

Aerial mycelium: None.

Soluble pigment: None.

4. Egg-albumen agar.

Growth: Moderate, thin and spreading, deep in medium; color close to dawn gray (10).

Aerial mycelium: White, with patches of bluish green aerial mycelium scattered throughout.

Soluble pigment: None.

5. Dextrose nitrate agar.

Growth: Moderate, in deeply wrinkled colonies, deep in medium; color close to cream (10).

Aerial mycelium: Trace of white aerial mycelium appearing late.

Soluble pigment: Trace, light-brown.

6. Tyrosinate agar.

Growth: Scattered, in the form of slightly wrinkled colonies, deep in agar.

Color: close to argus-brown (10).

Aerial mycelium: None.

Soluble pigment: None.

7. Glycerin nitrate agar.

Growth: Moderate, in form of wrinkled colonies, deep in medium. Color, close to cream (10).

Aerial mycelium: None to scant white patches.

Soluble pigment: None.

8. Glycerin asparaginate agar.

Growth: Moderate, scattered in form of colonies, deep in medium.

Aerial mycelium: None to scant patches of white and bluish-green aerial mycelium.

Soluble pigment: None.

9. Calcium malate agar.

Growth: In the form of irregular colonies, deep in medium.

Aerial mycelium: Small amount, white to gray.

Soluble pigment: None.

10. Starch agar.

Growth: Moderate, smooth, deep in medium, ivory color.

Aerial mycelium: White, with patches of bluish-green aerial mycelium scattered throughout the white.

Soluble pigment: None.

Enzymatic zone: Complete hydrolysis after 12 days on 9-cm. Petri dishes.

11. Cellulose agar.

Growth: No growth.

12. Synthetic solution (saccharose).

Growth: Moderate, all in bottom of tube. Growth in form of white flakes.

Aerial mycelium: None.

Soluble pigment: None.

13. Dextrose broth.

Growth: Moderate, all in bottom of tube, flaky.

Aerial mycelium: None.

Soluble pigment: None.

14. Synthetic solution (glycerin).

Growth: Scanty, flaky, mostly in bottom of tube, few suspended colonies.

Aerial mycelium: None.

Soluble pigment: None.

15. Potato plug.
Growth: Moderate, light brown, shiny, wrinkled.
Aerial mycelium: None.
Soluble pigment: None.
 16. Gelatin, 20° C., 25 days.
Growth: Scanty.
Aerial mycelium: None.
Soluble pigment: None.
Liquefaction: Medium to good, about $\frac{1}{4}$ of gelatin in tube liquefied after 25 days.
 17. Milk.
Growth: In form of ring.
Hydrolysis: The milk was hydrolyzed, without visible coagulation. Hydrolysis began to show after 6 days and at the end of 35 days about $\frac{1}{2}$ of each tube was hydrolyzed.
- III. Biochemical Features.
1. Nitrite formation: Faint on saccharose synthetic solution; fair on glycerin synthetic solution and nitrate broth.
 2. Proteolytic action: Fair in milk and gelatin.
 3. Change of reaction: Changed to acid reaction in dextrose broth and saccharose synthetic solution; no change in gelatin and glycerin synthetic solution.
 4. Diastatic action: Excellent in tubes and on plates.
 5. Growth on cellulose: None, with methods used.
- Hab. Isolated from diseased sweet-potato tubers and small rootlets, from several localities in Louisiana.

SUMMARY

In certain districts in Louisiana in recent years soil rot of sweet potatoes has been spreading rapidly and is becoming a very serious disease.

The soil-rot organism has been isolated and is described as a new species, *Actinomyces ipomoea*.

A disease with symptoms somewhat different but similar to those originally described by Halsted for the soil rot or pox disease also occurs in Louisiana. The relation of this disease to the one that is so destructive has not yet been definitely determined.

Soil rot is more serious in dry soils and in dry seasons.

The disease is ordinarily found in soils with the pH above 5.2.

A method of testing the virulence of isolates of *Actinomyces* in the laboratory is described.

The disease with typical symptoms has been produced in the greenhouse and in the field in inoculation experiments with pure cultures of *Actinomyces ipomoea*.

In cultures the optimum temperature of growth for *Actinomyces ipomoea* is in the neighborhood of 32° C.

The optimum hydrogen-ion concentration for growth is pH 5.6 or higher. When the hydrogen-ion concentration of the soil in the field is lowered to pH 5.0 with an application of sulphur, there is very little development of soil rot.

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THE HISTOLOGY OF INFECTION OF SUSCEPTIBLE AND RESISTANT SELFED LINES OF RYE BY THE RYE SMUT FUNGUS, *UROCYSTIS OCCULTA*¹

LEE LING

(Accepted for publication May 2, 1940)

Selfed lines of rye vary greatly in their resistance to the rye smut fungus, *Urocystis occulta* (Wal.) Rab., as Stakman, Moore, and Cassell (8) reported after studying the reactions of 139 selfed lines of rye in Minnesota. The selfed lines they tested ranged from highly resistant to completely susceptible, but they found no evidence of physiologic specialization of the pathogen. Although Wolff (14) very early studied infection of the rye coleoptile by the germinating sporidia of *U. occulta*, it was not known whether the early stages of the infection were the same in resistant lines of rye as in the susceptible lines.

To determine whether rye smut resistance is due to inhibition of chlamydospore germination, to prevention of fungal entrance, or to some other cause, 8 selfed lines of rye were obtained from the Division of Agronomy and Plant Genetics, University Farm, St. Paul, Minnesota, and inoculated with rye-smut chlamydospores. One line was completely susceptible in the field, three were moderately susceptible, and four were resistant. The inoculated seeds were germinated at 15° C. in glass dishes lined with wet cotton. After 2½ days, chlamydospores on the young seedlings were washed off in drops of water and the percentage of chlamydospore germination

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determined. After 6 days the outer and inner epidermises were stripped from the coleoptiles of the seedlings and examined microscopically for hyphae. There was no significant difference in percentage of chlamydospore germination on seedlings of the resistant and susceptible host groups (Table 1), while mycelium was present on all, or nearly all, 6-day-old seedlings in both groups; hence, chlamydospore germination was not inhibited by resistant plants and the initial phases of infection seemed to be the same in resistant and susceptible lines of rye.

TABLE 1.—*Reaction of 8 selfed lines of rye to Urocystis occulta*

Selfed lines, 1935 culture number	Percentage of infection in field (1934-35) ^a	Percentage of germinated chlamydospores on 2½-day-old seedlings	Microscopic examination of 6-day-old seedlings	
			No. examined	No. free from mycelium
C-51	100	34	25	0
C-26	89	18	15	0
C-35	69	22	15	0
C-7	61	35	15	1
C-28	0	17	25	0
C-37	0	29	15	1
C-65	0	16	15	2
C-85	0	18	15	0
(Rosen) ...	12	15	0

^a Data were supplied by M. B. Moore, of the Division of Plant Pathology, University Farm, St. Paul, Minnesota.

The mode of penetration and the mycelial development were studied in detail in only 4 of the selfed lines: the very susceptible line C-51, the susceptible C-26, and the resistant lines C-28 and C-37. Inoculated seeds were germinated at 15° C. on wet cotton, and after the first foliage leaf emerged from the coleoptile the seedlings were transplanted to sterile sand. Fresh material, obtained by stripping the epidermis from the young seedlings, was examined daily. It was mounted in water without staining, or in lacto-phenol after staining with cotton blue in lacto-phenol. Selected portions of seedlings, including the growing point, also were fixed 3, 4, and 6 days after planting and at 1-week intervals thereafter. Craf's fixative, together with the process of dehydration and imbedding described by Randolph (7), was generally used because of its simplicity and was quite as satisfactory as Navashin's. Sections were cut from 8 to 12 μ thick. Numerous stains and combinations were tried, but crystal violet, Gram's iodine, and light green gave the best results. Picric acid was very satisfactory for destaining and differentiation, as it readily removed the purple color from the cytoplasm, thus making the nuclei more distinct.

Woolman (15) used the reaction to Gram's stain as one means of distinguishing different phases in the infection of wheat by *Tilletia tritici* (Bjerk.) Wint., the hyphae in the first phase being gram negative. In *Urocystis occulta* the situation was somewhat comparable, but the distinction between different stages by staining reaction was not so clear, and was less

reliable than in Woolman's work. Penetrating hyphae usually are easily recognized, as the host cells in the immediate vicinity ordinarily become markedly purple with the staining combination used. The infection hyphae, however, are visible because of the transparency of crystal violet. Although the hyphae do not stain well while restricted to the outer layers of cells in the coleoptile, they can, however, be followed because of the deeply stained nuclei. In the true leaf, especially in the meristematic region, the mycelium may become heavily stained, but the nuclei of the hyphae often could not be satisfactorily differentiated.

PENETRATION BY THE PARASITE

Wolff (4) stated that infection normally takes place through the coleoptile anywhere from 8 to 10 mm. above the root node. The writer also found penetrations only above the coleoptilar node, the earliest entrance of the pathogen being observed on a seedling three days old.

The infection hyphae were binucleate, although general conclusions regarding the origin of the dikaryophase could not be drawn. In no case in the present study, however, was the infection hypha observed arising from fused sporidia. On the contrary, the hyphae often were found still attached to chlamydospores lying outside the epidermis (Fig. 1, A). Although penetration by such infection hyphae may not be the only method of entrance for the pathogen, it undoubtedly is common. In a study of *Ustilago avenae* (Pers.) Jens., Western (12) reported that under natural conditions the infection tube either is the promycelium itself or arises from the fusion of adjacent promycelial segments, while the sporidia are produced much less abundantly than in artificial culture. Thus the paired nuclei are derived from a single diploid nucleus; consequently, the opportunity of establishing new parasitic races through hybridization is restricted. In *Urocystis occulta* the situation is somewhat comparable. As pointed out by Stakman, Cassell, and Moore (8), the sexual process in *U. occulta* is more or less analogous to self-fertilization in higher plants. The haplophase is reduced to the sporidia, which remain attached to the parent promycelium and have very little opportunity for fusion with sporidia that are not actually sister sporidia. The writer (5) experienced great difficulty when he attempted to isolate sporidia and culture monosporidial lines, for the sporidia of rye smut do not function as conidia. Therefore, it is relatively unimportant whether sporidia are produced, as nuclear recombination is limited principally to the haploid derivatives of a single diploid nucleus, and it matters little whether recombination occurs directly in the promycelium or through the fusion of sporidial branches. The possibility of wider crossing between sporidial branches from different promycelia is, however, eliminated when sporidia are not formed. The functioning of the promycelium as an infection hypha may partly explain the remarkable uniformity in pathogenicity of many collections of this fungus.

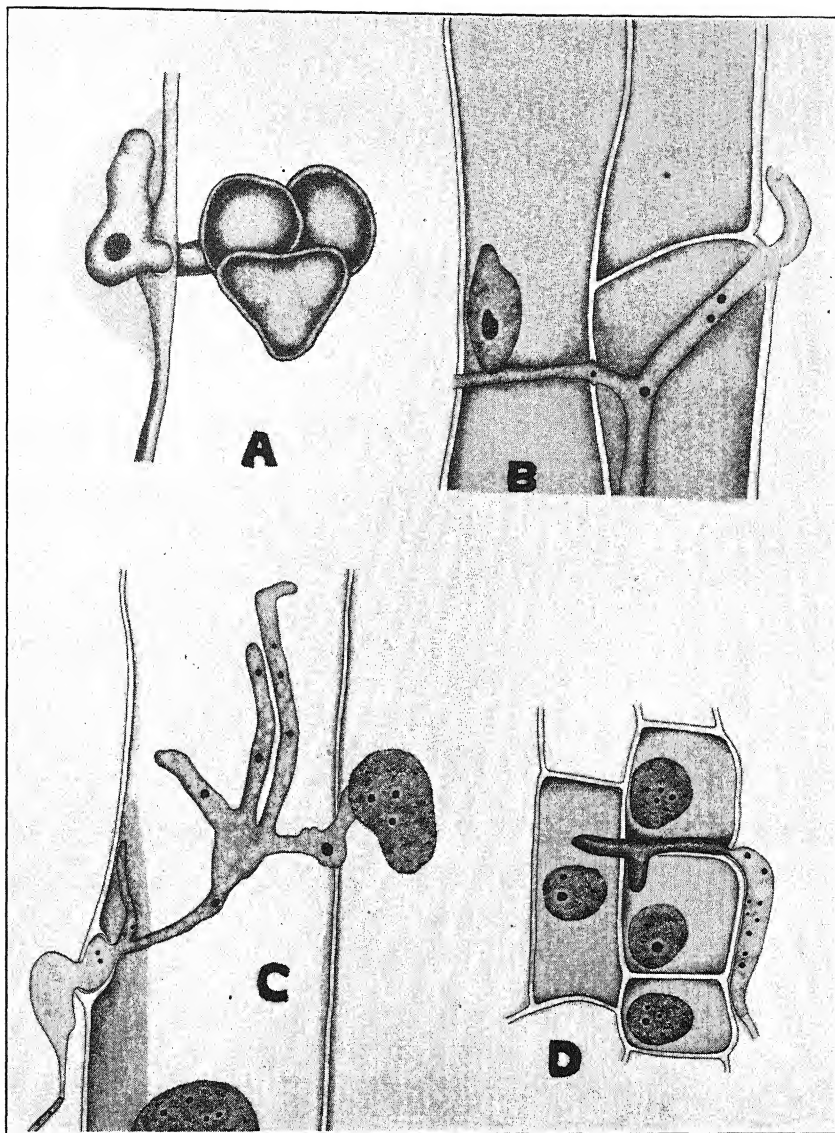


FIG. 1. A. Infection tube originating directly from a chlamydospore on the epidermis of a resistant seedling 14 days old. Note the thickening on the inside of the cell wall of the host. B. Penetration and early development of *Urocystis occulta* in a susceptible seedling 4 days old. Note the apparent dissolution of cell wall around the point of entry. C. Similar to B. Note the appressorium-like structure and also the knob-like enlargement within the epidermis. D. A multinucleate hypha with a constriction penetrating through the second leaf of a susceptible seedling 14 days old. All $\times 1580$. The drawings for figures 1 and 2 were made by William H. Lindemann.

The early development of the pathogen was similar in both resistant and susceptible lines, the coleoptiles of both types being penetrated by the infection tube with equal facility during the 4–6 days following seed germination. The first indication of infection was a thickening of the inside of the epi-

dermal wall. In a few instances, a curvature on both sides was noticed. A common type of infection is illustrated in figure 2, A, and also in figure 3, A, in which the infection hypha pushes the epidermal wall inward to form a large, deep cavity. In the tangential view in figure 2, B, the hypha has penetrated the epidermis at the bottom of the cavity and is surrounded by a dense cytoplasmic layer inside the host. A constriction in the hypha is

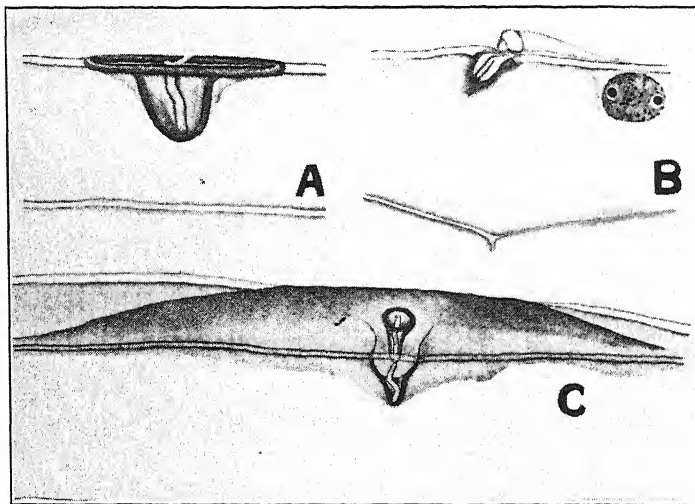


FIG. 2. A. Initial stage of infection of a 6-day-old susceptible rye seedling by *Urocystis occulta*. The epidermal cell wall of the coleoptile has been pushed inward by the infection hypha to form a deep cavity. B. Hypha penetrating the first leaf of a resistant seedling 21 days old, showing an appressorium-like structure. C. A tangential section of a 6-day-old resistant seedling including a portion of epidermal surface. The infection hypha has penetrated the epidermis and has been surrounded by a funnel-like structure inside the host. The invagination of epidermal cell wall can be seen in top view. All $\times 850$.

visible at the point of entrance. Judging from the staining reaction, the process of penetration apparently is not only mechanical, but also chemical in nature. As pointed out by Brown (2), the chief objection to the chemical theory of penetration is that there has been no demonstration of a chemical substance capable of dissolving the cuticle. In the present study, however, the infection ordinarily takes place before the epidermis has become heavily cutinized. Even in the more advanced stage of cutinization, the wall material beneath the cuticle may be acted upon by a fungus secretion and thus become softened and gelatinized. This, together with the pressure exerted by the hyphal tip, enables the fungus to make its way through the relatively thick epidermis. The whole process is comparable to a stretched gelatinized sheet pressed inward by a stick. Brown also criticized staining reaction as inadequate evidence for dissolving of the cuticle. As long as there is no evidence that a similar reaction could be produced by mechanical force, a change in staining reaction should be admitted as an indication of chemical change in the host in response to the fungus activity.

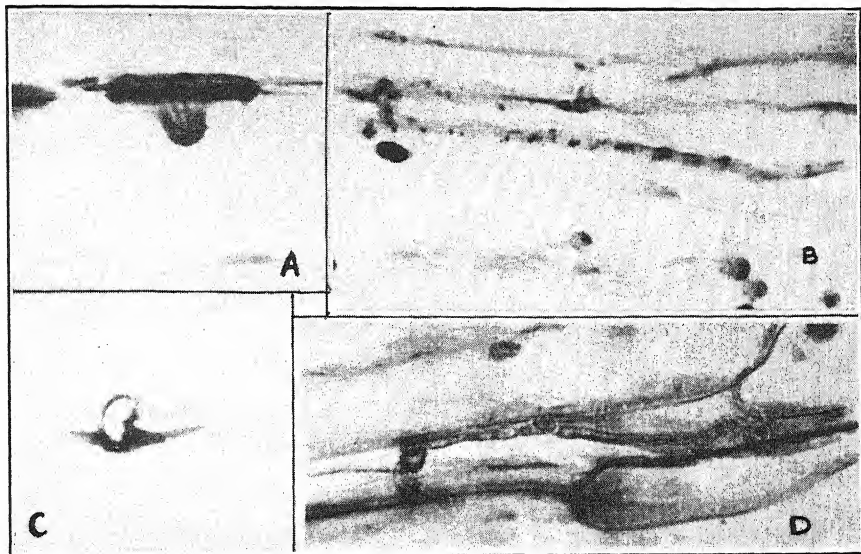


FIG. 3. A. Photomicrograph of material drawn in figure 2. A, showing the deep cavity formed by an infection hypha in the epidermal cell wall of the coleoptile of a 6-day-old susceptible rye seedling. Approx. $\times 650$. B. A binucleate intercellular hypha in the coleoptile of a 6-day-old susceptible rye seedling. Approx. $\times 800$. C. Hypha with a peg-like tip attempting to penetrate a wall between cells in the coleoptile of a 6-day-old resistant rye seedling. Approx. $\times 1500$. D. A long, vacuolate hypha in the coleoptile of a 14-day-old resistant rye seedling. Approx. $\times 800$.

It was not uncommon to find infection hyphae making their way into host cells without inducing such conspicuous changes. In figure 1, A, the thickening of the inner epidermal wall surface seems to be the only change in the host. Figure 1, B, indicates that the invaded cell was not much disturbed, except that the cell wall appeared to dissolve in the immediate vicinity of the entrance. In both cases the diameter of the infection hypha was unchanged, this observation not being in complete agreement with an hypothesis of mechanical penetration. An appressorium-like structure was often observed at the place of contact with the host (Fig. 1, C, and 2, B). Figure 1, C, also shows an enlarged portion of the hypha within the epidermal wall.

Fungus penetration varies slightly with the age at which seedling infection occurs. During the first 4 to 6 days the penetration processes are similar in resistant and susceptible hosts. Occasionally, even after 6 days, the fungus has difficulty entering the coleoptile. In older seedlings infection may occur in the tissues of the first or second leaves as well as in the coleoptile, but penetration of cell walls may be more difficult than in young seedlings. (See figure 2, B, for infection of a seedling 21 days old.)

DEVELOPMENT OF FUNGUS IN SUSCEPTIBLE LINES

Once the fungus is inside the host it branches profusely, extending into both the inter- and intracellular spaces. The progress of the mycelium in a very susceptible line, C-51, is diagrammatically shown in figure 4. In

4-day-old seedlings (a) the mycelium ordinarily is restricted to the outer layers of cells in the coleoptile; in one case only was it found in the space between the coleoptile and the first leaf. The fungus grows in all directions and advances very rapidly transversely toward the deeper tissues. Within 6 days the hyphae had reached the inner epidermis of the coleoptile (Fig. 4, B). Hyphal segments were observed in the space between the coleoptile

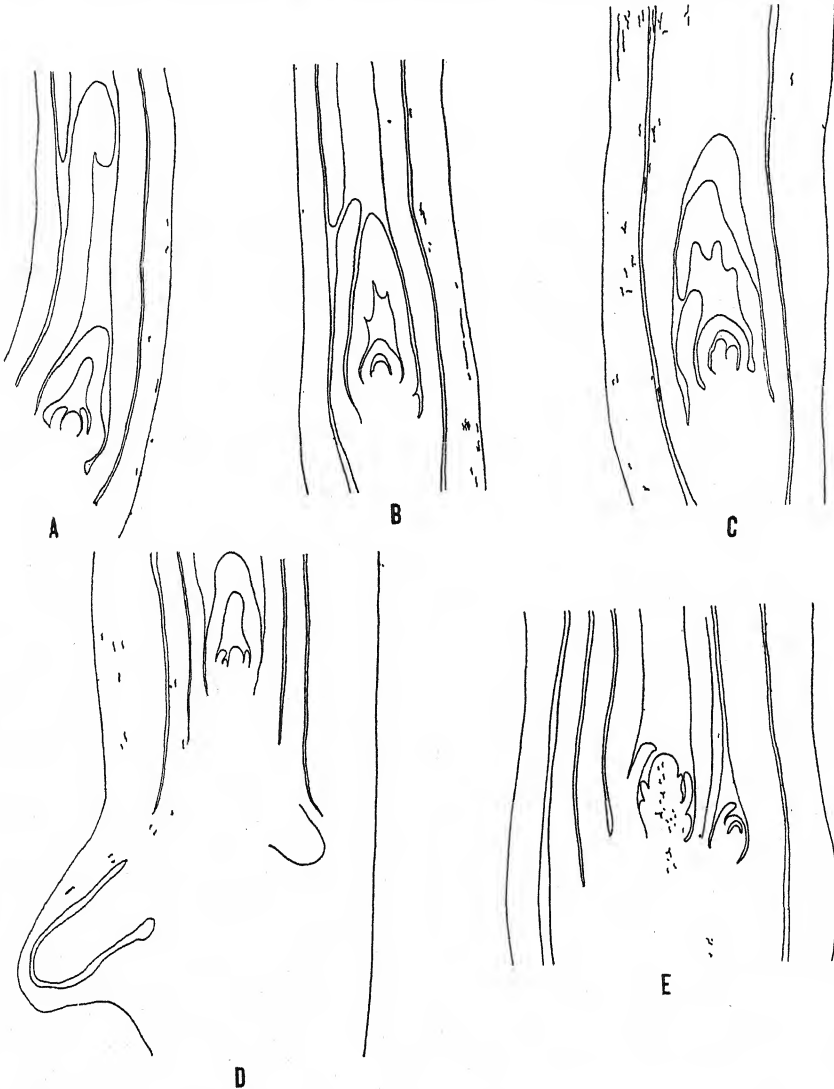


FIG. 4. Diagram showing progress of *Urocystis occulta* in susceptible seedlings of C-51 line of rye. Mycelium indicated by short lines. A, 4 days; B, 6 days; C, 14 days; D, 21 days; E, 35 days. Approx. $\times 20$.

and the first leaf. The nuclear condition of the fungus was best observed in these earlier stages, both the inter- and intracellular hyphae being binucleate (Fig. 3, B), with the nuclei paired in a very regular way.

In 14-day-old seedlings (Fig. 4, C) hyphae were observed in the first leaf, more abundantly toward the tip. The hyphae usually were fairly long and often much branched. Scattered pieces of mycelium were observed in the second leaf. Figure 1, D, shows a multinucleate hypha with nuclei apparently still paired, working its way into the second leaf. The smut never was found in the coleoptilar internode until the plants were 21 days old (Fig. 4, D). At 28 days the hyphae, which were essentially intercellular, had spread into the meristematic region. But the invasion was completed only after 35 days, when the greatly contorted hyphal segments were seen abundantly in the growing point (Fig. 4, E).

DEVELOPMENT OF FUNGUS IN RESISTANT LINES

The earlier phases of infection in resistant lines did not differ from those in susceptible ones. (Compare A and C of Fig. 2.) The mycelium was equally abundant in the two or three outer layers of cells in the coleoptile at 4 days, but the progress was greatly retarded thereafter. In 14-day-old resistant plants the mycelium had not progressed so far as in 6-day-old susceptible plants. It was not until 21 days that the hyphae were observed beginning to penetrate the first leaf. Figure 2, B, illustrates such a penetrating hypha enlarging at the point of contact with the host and forming a funnel-shape structure inside the epidermis. However, the penetration appeared to have no apparent effect on either the host or the fungus itself. In 28-day-old plants scattered hyphal segments still could be seen in the first leaf, but no further progress was noted.

A hypha often had a peg-like tip when passing from cell to cell in the coleoptile (Fig. 3, C). Further indication that there might be some mechanism retarding their development was the confinement of some of the seemingly healthy hyphal pieces in the epidermal layer, even after 14 days. At this stage a few hyphae appeared vacuolated (Fig. 3, D).

HOST-PATHOGEN RELATIONSHIP

In connection with her study of *Ustilago avenae*, Kolk (4) stated: "Compatibility between host and pathogen reaches such a high degree of development that the fungus causes no change in the appearance of the cells . . . and there is little difference between cells invaded by hyphae and those not." This statement is in agreement with most investigations on smut and also applies to *Urocystis occulta*. In the present study the host-pathogen complex seemed to approach a high degree of harmony, with very little deleterious effect to either organism. Necrosis of host cells in advance of the hyphae, as described in corn invaded by *Ustilago zeae* (Beckm.) Ung. (9) and in Markton oats invaded by *U. avenae* (11), was not observed. In resistant lines of rye, especially C-28, numerous small, light-green spots were found at the base of the first leaf 21 days after infection, the time of penetration of the first leaf by the fungus. However, these cells did not stain abnormally.

Walter (9) observed that the germ tubes of *Ustilago zeae* often seemed to be attracted to the nuclei of host cells. In the present study the infection hyphae also were sometimes, but by no means always, seen in association with host nuclei (Fig. 1, B and C), but neither the cytoplasm nor the nuclei of host cells seemed to respond in any consistent way to the presence of the fungus. Nor was any indication found of a sheath such as illustrated by Wolff (14).

The presence of haustoria has been reported in three species of *Urocystis*: *U. occulta* (14), *U. cepulae* Frost. (1, 13), and *U. anemones* (Pers.) Wint. (6, 10). In Wolff's illustrations (14), however, the slender branches sent into host cells by intercellular hyphae appear more like ordinary intracellular hyphae than true haustoria. If an haustorium is considered to be a specific absorbing organ with limited growth and definite form characteristic of one species, then haustoria probably do not exist in *U. occulta*. Although it was not uncommon to find simple knob-like or coralloid bodies inside the cells of the coleoptile and in the first leaf, these may be considered simply the intracellular hyphae rounded up and massed in the cell cavities.

It was frequently observed that host cells took the purple stain, possibly indicating a chemical change in their cytoplasm. Another commonly observed effect of the fungus on the host was the enlargement of intercellular spaces by the action of mycelium.

Owing to the presence of a great mass of smut mycelium, portions of rye coleoptile frequently underwent a slight disorganization, thus paving the way for certain secondary organisms. Thus, one side of the coleoptile of a plant 14 days old was entirely destroyed by a secondary parasite, while the other side remained normal. On close examination, it was apparent that smut mycelium had been present only in the one side of the coleoptile that became disorganized. This gives a reasonable explanation of the finding of Geach (3), who reported that wheat seedlings infected with flag smut were predisposed to the attack of *Fusarium culmorum* Sacc.

SUMMARY

The resistance of rye to *Urocystis occulta* does not seem to be due to any inhibition to germination of smut chlamydospores on resistant varieties, because chlamydospores germinated equally well on seedlings of all the selfed lines of rye and produced mycelium on all.

The smut fungus penetrates resistant lines of rye as well as susceptible ones, so that the early stages of smut infection appear similar in all host lines. There are slight variations in the reaction of a host to an infection hypha, especially as the age of the rye seedling increases, but there are no consistent differences between resistant and susceptible lines of rye in this respect during the first 6 days after seed germination.

A binucleate infection hypha commonly penetrates directly the epidermal cell wall of the rye coleoptile. The process seems to be partly mechanical, partly chemical. Sometimes the host cell wall changes very little except

for a slight thickening and softening of the wall at the point of entry. At other times the host cell wall is greatly thickened and probably changed chemically, so that the inner epidermal cell wall is greatly stretched and definitely invaginated by the pressure of the fungus hyphal tip. The hypha itself may or may not be constricted as it enters a host cell.

Mycelial development is rapid and abundant in a susceptible line of rye, the smut hyphae being found in the coleoptilar internode about the time a seedling is 21 days old, and in the meristematic region when the seedling is 28 days old. Mycelial development in a resistant line of rye was meager and retarded in spite of the fact that infection hyphae readily penetrated such a host. Scattered hyphal segments occur in the outer layers of coleoptile and first leaf, but the mycelium does not spread to the meristematic region. Some of these hyphal segments remain healthy, while others become vacuolate.

The rye smut fungus, *Urocystis occulta*, resembles *Tilletia tritici* and *Ustilago avenae* in that its early stages of infection are alike in susceptible and resistant hosts.

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WOOD DECAY IN APPLE TREES IN MINNESOTA¹

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The short life of apple trees in many regions of the world has been attributed perhaps chiefly to adverse climate and unfavorable soil, although the occurrence of decay in declining trees has been observed in India (1, 6, 10), Australia (2, 15), Great Britain (5, 17), South Africa (8, 11), and various parts of the United States (7, 9, 13, 18). Obviously, any attempt to increase the productive lives of these trees by breeding longer-lived strains or by other means must take into account the factors involved in their decline. The observations recorded in this paper suggest that, in Minnesota, wood decay may be one, and possibly a fairly important, cause of senescence of apple trees, especially in combination with sunscald and cold injury.

Brierley (4) states that the average life of an apple tree in Minnesota is 30 years. An average tree begins to bear when 6 to 8 years old, reaches maximum production when 20 years old, and thereafter gradually fails. He expresses the opinion that the short life of apple trees in this region is due primarily to the severe winters. Steinmetz and Hilborn (14) state that, in Maine, blackhearted wood caused by low temperature is more susceptible to decay than uninjured wood. Bradford and Cardinell (3), also, state that wood decay frequently follows winter injury.

In 1936, 1937, and 1938 the writers felled and sectioned approximately 150 apple trees from 10 to 30 years of age, for the purpose of determining the incidence of rot, the means of its entry into the trees, its extent in each tree, its correlation with sunscald, cold injury, frost cracks, and other injuries, and its apparent effect on the health and life of the trees. Fruit bodies of wood-rotting fungi were collected from decayed trees, identified, and cultured, and these cultures were used as a basis for identifying fungi isolated from decayed trees on which no fruit bodies were present.

INCIDENCE OF ROT

Rot of limited extent was found in 48 out of 50 15-year-old seedling trees that had been derived from a number of crosses. These trees averaged 9.5 cm. in diameter, and the rot column, at a height of 3 feet above the ground, averaged 2.4 cm. in diameter. These trees were grown only 4 feet apart in the row, and the consequent crowding may have affected their susceptibility to decay. Sixty-eight trees 22 years old in one orchard were cut down and sectioned. Of these, 12 were badly decayed, the rot extending throughout most of the diameter of the trunk and out into some of the branches. Of the remainder, 22 were moderately decayed, 22 slightly decayed, and no decay was found in 12, although a small amount of rot could have been

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present without having been detected. A smaller number of trees from 20 to 30 years old were cut in several other orchards at the University Farm, St. Paul, at the University Fruit Breeding Farm, Excelsior, and at Lake City, Minnesota. Decay was fairly extensive in most of these, involving a greater volume of wood in older trees than in young ones, and in trees that were dying the rot often extended out to or even through the bark over a part of the trunk.

AMOUNT OF ROT IN DIFFERENT VARIETIES

The small number of trees of each variety that were dissected made it impossible to judge accurately the relative susceptibility of the different varieties to decay. However, the limited observations made indicate that the incidence and extent of rot at a given age are greater in Wealthy than in Oldenburg, Hibernial, or Patten Greening. This agrees with Brierley's (4) estimate that the average life of Wealthy trees in Minnesota is about 26 years, while the others live 34 to 37 years.

MEANS OF ENTRY

In by far the greater number of cases whose origin could be definitely traced, rot had entered through branch stubs. Stubs no more than $\frac{1}{4}$ in. in

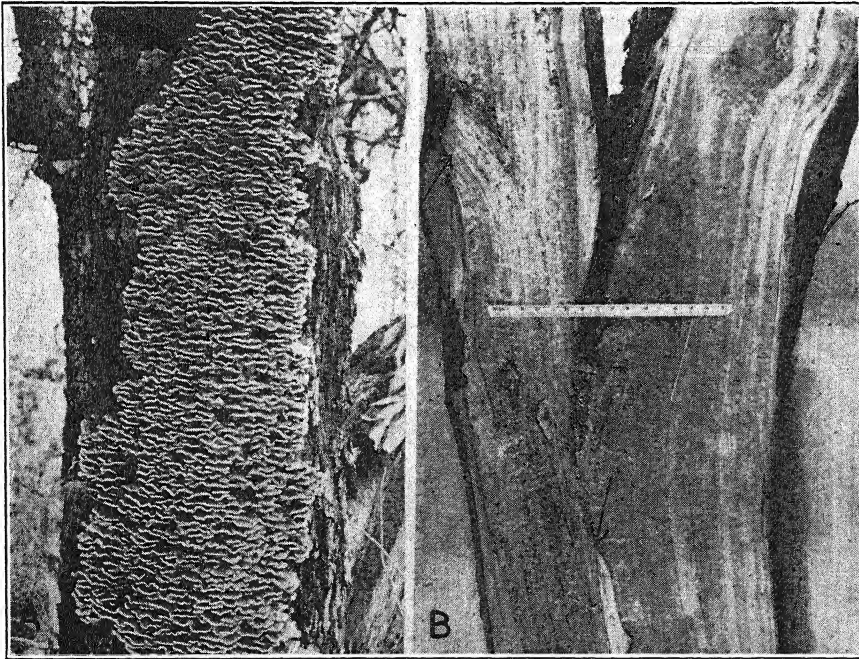


FIG. 1. A. *Polyporus versicolor* fruiting on an old apple tree. The fruit bodies extend laterally beyond what appeared to have been originally a sunscald injury. All the interior of the tree was rotted and only a thin, narrow strip of cambium and bark remained alive. B. Section of a 22-year-old decayed tree showing entrance of rot through a branch stub about 1 inch in diameter and also through a partially healed crotch crack.

diameter permitted decay to enter, although if these healed over quickly the decay progressed only very slowly. Rot entering through stubs an inch or more in diameter usually continued to advance fairly rapidly, especially if the wood at the end of the stub was so thoroughly decayed before the callus layer grew over it that the latter had no foundation on which to grow and thus failed to close over the wound completely. Stubs two or more inches in diameter invariably were rotted (Fig. 1, B). The origin of several cases of rot was traced to split crotches, and some rot entered through

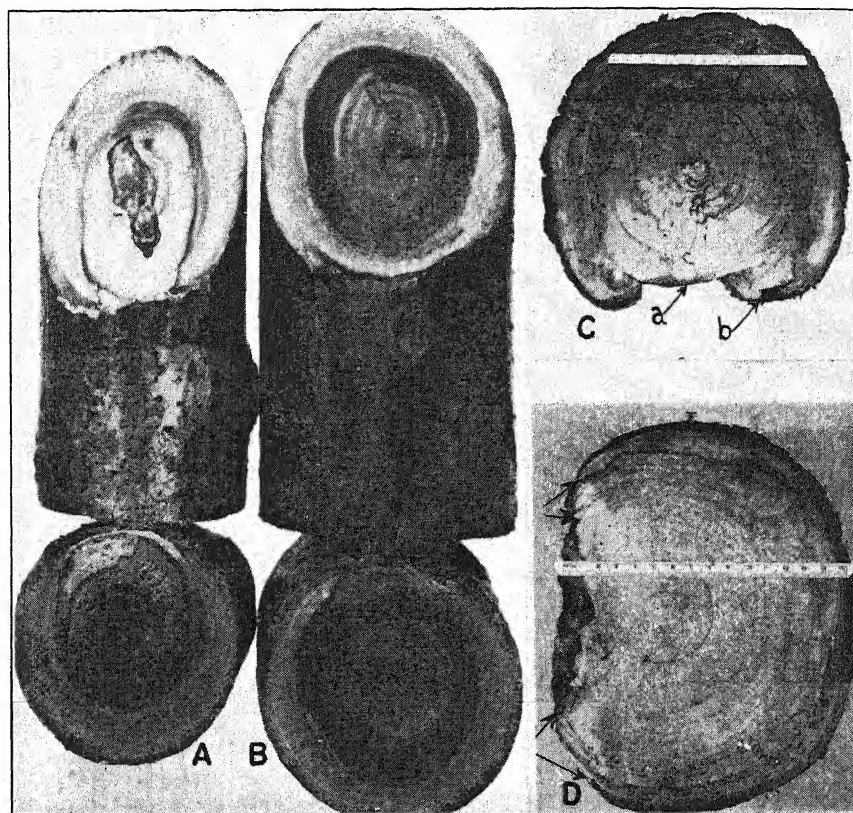


Fig. 2. Rot was present in A and B before portions of the trunks were injured by sunscald. In A the rot has progressed out to the injured portion. Incipient rot was present in B, as indicated by the pale region near the center of the tree, and this rot has extended out toward and reached a more advanced stage near the sunscald. Decay also is entering through the sunscald. The dark areas are typical of cold injury. In C the rot entered through a sunscald injury at *a*. At *b* the rot has progressed out to the bark and it is unlikely that such a wound could heal over. D is a cross section of an old, thoroughly decayed tree. Either the wood-rotting fungus or other agents have killed the tissue at the border of several successive callus layers, as indicated by the arrows. The tree obviously was failing rapidly when cut.

frost cracks and through sunscald injury (Fig. 2, C). Rot was found to have entered through the roots of 3 of about 50 grafted trees 22 years old whose roots were examined thoroughly. No rot was found to have entered

through the roots of the 25 seedling trees 15 years old whose roots were pulled up and examined.

CORRELATION OF ROT WITH INJURY

It has been observed frequently (3, 14) that wood decay apparently follows winter injury. Our observations support Steinmetz and Hilborn's (14) statement that the dark wood resulting from low-temperature injury is more susceptible to rot than uninjured wood. They also found that trees without blackheart succumbed to fungus disease. We observed that the dark wood frequently extended out into branches $\frac{1}{4}$ in. or less in diameter and almost always preceded the invasion of these branches by rot. We were not able to get any evidence that the presence of rot predisposed trees to further cold injury, but neither was it possible to see that cold injury by itself was especially injurious to the trees. Steinmetz and Hilborn (14) found that if less than 20 per cent of the vessels in the secondary xylem were occluded and if less than 25 per cent of the parenchyma cells were killed by cold injury, the tree or branch would probably recover. If, however, over 50 per cent of these tissues were affected death usually followed.

The occurrence of general and unusually severe sunscald early in 1936 offered an excellent opportunity to observe the influence of this type of injury on decay. Figure 2, A and B, shows a typical example of what was found. Where decay already was present in the trunk of a tree, the death of the bark and cambium resulting from sunscald permitted the rot to spread rapidly out toward this injured or killed tissue. It undoubtedly also facilitated the longitudinal spread of decay, although this could not be determined in the trees examined. Once the rot had reached the cambium region it spread laterally very slowly, growing in tissue killed in succeeding years at the border of the original injury. It probably also predisposed the border tissue to further injury and thus retarded or prevented healing (Fig. 2, C). A few cases were found, in old trees, in which the fungi causing rot appeared to have acted as parasites, gradually killing the cambium and inner bark around the periphery of the trunk, even on the side where no sunscald could have occurred. In such cases it is almost unquestionable that rot was a major final factor in the decline of the tree, whatever the original factors may have been. Such a tree is illustrated in figures 1, A and 2, D.

IDENTITY OF THE FUNGI CAUSING ROT

Several hundred isolations were made, chiefly on malt and potato-dextrose agar, and a large number of different fungi were obtained, only a few of which could be identified with reasonable certainty. Where fruiting bodies were found on the trees and cultures of the same fungus, but of no other fungi, were obtained from different portions of the rot within the tree, it seemed reasonable to consider that fungus chiefly responsible for the rot in that particular tree. Sometimes two or more fungi were isolated

from the rot in a single tree; and in many cases when fungi were isolated from trees on which no fruit bodies were present, it was impossible to identify them. Various species of molds were isolated from the advanced decay, as would be expected, and bacteria were isolated from a large proportion of the decayed trees. The possible effects of these latter on the trees was not investigated. The wood-rotting fungi that were identified are presented in table 1.

TABLE 1.—*Wood-rotting fungi isolated from decaying apple trees*

Species	Means of identification
<i>Fomes applanatus</i> (Pers.) Wallr.	Comparison of macroscopic and microscopic characters of cultures from decayed wood with cultures from sporophores on the same tree.
<i>Trametes malicola</i> Berk. and Curt.	do
<i>Polyporus versicolor</i> (L.) Fries	do
<i>Thelephora</i> sp.	do
<i>Polyporus resinusus</i> (Schrad.) Fries	Comparison of macroscopic and microscopic characters of cultures from decayed wood with cultures from fruit bodies on basswood trees.
<i>Pholiota adiposa</i> Fries	do. (Fruit bodies of <i>P. adiposa</i> were present on the decayed apple tree, but were too decomposed to permit culturing.)
<i>Schizophyllum commune</i> Fries	Formed fruit bodies in culture.
<i>Trametes hispida</i> Bagl.	do. Also comparison of macroscopic and microscopic characters of cultures from decayed wood with cultures from fruit bodies on the same tree.
<i>Polyporus adustus</i> (Willd.) Fries	Fruit bodies appeared on decayed logs placed in moist ground.
<i>Lenzites betulina</i> (L.) Fries	do

Besides the fungi identified, a large number of isolates were obtained that could not be identified, although the cultures were compared with those obtained from fruit bodies on apple trees and with cultures from fruit bodies of fungi that were common on other deciduous trees. These unidentified cultures could be divided, on the basis of macroscopic and microscopic characters, into about 7 groups; but, of course, it was not known that such a division would correspond to taxonomic groups, even though some of the cultures in each group obviously were identical. In any case, it is evident that a rather large number of species can inhabit and decay the wood of living apple trees. No one of the species nor any certain combination of them was found often enough to be considered responsible for a major part of the decay.

These observations support published reports in the conclusion that a relatively large number of fungi are capable of causing wood decay in apple trees. Seymour (12) lists many hymenomycetes as occurring on the apple, but not all of these are known to cause decay in living trees. Hesler and Whetzel (7) mention *Fomes igniarius* (Fries) Gillet as the cause of heart

rot; *Polyporus versicolor* is reported as the agent of wood decay by Thomas (15), Birmingham (2), Horne (9), and others. Brooks (5) found *Polyporus adustus* apparently causing a destructive decay of apple trees in England, while Smith (13) describes *Trametes hispida* as being responsible for the death of numerous apple trees in Colorado. Wormald (17) cites *Polyporus hispidus* (Bull.) Fries as a cause of heart rot in apple, and *Stereum purpureum* Pers. invades apple wood, causing silver leaf (17).

Schizophyllum commune is probably most frequently mentioned as a cause of wood decay in fruit trees, several authors (1, 2, 4, 8, 10, 11, 16) having recorded the occurrence of fruiting bodies on apple trees. Some of these reports state that it is a saprophyte on the bark (11) or a secondary invader (16). Others do not make clear to what extent it is pathogenic, while Birmingham (2) states that it causes heart rot, killing numbers of reworked apple, cherry, and plum trees. Chaudhuri and Johar (6) found by inoculation that *S. commune* could penetrate and discolor the wood of living apple trees, as well as other species. Narasimhan (10) inoculated apple trees with a pure culture of the fungus, but got no infection. Putterill (11) did not inoculate apple, but found by inoculation that *S. commune* could cause decay in peach and almond. Putterill isolated the fungus from decayed peach and almond wood and also from dead apple bark where it was "growing as a saprophyte." The "saprophytic" strains did not fruit in culture so readily as the parasitic ones.

Sporophores of *Schizophyllum commune* are abundant in Minnesota on apple bark killed by sunscald. It was isolated from wood about 2 mm. below the surface of the bark that bore sporophores, and once from the wood of a small branch. Of all the other isolations made, no cultures were found that produced the sporophores of *S. commune* or resembled it in culture. Apparently, in Minnesota, this fungus is a secondary invader of dead bark, but not an important cause of wood decay in apple.

Other species of fungi, including *Polyporus tulipiferus* (Schw.) Overholts, *P. pubescens* (Schum.) Fries, *Lenzites trabea* (Pers.) Fries, and *Daedalia unicolor* (Bull.) Fries, were observed repeatedly growing on dead branches, but no evidence was found that they caused decay in living trees. *P. tulipiferus* and *D. unicolor* are rather common causes of decay in some other deciduous trees, and it is likely that a further search would prove that they decay apple trees also.

A particular type of decay was not associated with each species of fungus. In most cases, regardless of the fungus or fungi causing the rot, the decayed wood at first became permeated with radially elongate white, punky flecks, and eventually became uniformly white and punky in texture, although there was some variation in this. *Pholiota adiposa* and *Trametes malicola*, each of which was found once, were associated with a brown cubical rot.

PRODUCTION OF THE FRUIT BODIES

Attempts to induce the production of fruit bodies in the laboratory by inoculating sterilized blocks of apple wood in jars and large tubes were

mostly unsuccessful. *Polyporus versicolor*, *Trametes hispida*, and *Schizophyllum commune* produced several fruit bodies, *P. versicolor* when growing on wood, *T. hispida* when growing on wood or agar, and *S. commune* when growing on agar. The sporophores of the latter two produced spores in abundance, but those of *P. versicolor* did not. Abortive fruit bodies of an unidentified fungus, probably *Lenzites betulina*, also formed when the fungus was grown on sterile wood, but no others formed fruit bodies. Several sections of partly decayed trunks, each about 8 to 10 inches in diameter and 2 feet long, were set in pans sunk 8 inches in the ground, the upper end of each log covered with paraffin, and the ground kept saturated with water. Within about 6 weeks a heavy crop of fruit bodies of *Polyporus versicolor*, *P. adustus*, and *Lenzites betulina* appeared on these logs. The latter two species we had not previously observed on apple trees. It is possible that they may have infected the logs after these were set in the ground, but it is highly improbable that in so short a time they could have decayed enough wood to fruit so profusely. If, in further tests, this method of inducing the production of fruit bodies is found successful, it may be of considerable value in facilitating the identification of organisms causing decay in these trees.

DISCUSSION

Our observations indicate that rot enters apple trees in Minnesota at a fairly early period in their lives, chiefly through branch stubs, sunscald injuries, split crotches, and frost cracks, and becomes increasingly prevalent and involves a larger volume of the wood as the trees grow older. By the time the trees reach their maximum bearing age most of them are infected by wood-rotting fungi, and in many of them, even though they are no more than 20 to 25 years old, the rot extends throughout so great a portion of the tree that, even if it did no more than weaken the tree mechanically, there would be little chance that it would survive many years. This correlation of extensive rot with the gradual failure of the trees seems significant.

Once decay has invaded the tree, its spread seems facilitated by cold injury, sunscald, and frost cracks, all of which types of injury are common in this region. The fact that trees may suffer a certain degree of winter injury resulting in "blackheart" and yet recover, emphasizes the importance of the decay that follows such injury in hastening the decline of the trees. In the absence of either the decay or the predisposing effect of winter injury, it is probable that the trees would live much longer, and, consequently, the problem of senescence in this region is one involving both of these factors. Certainly our observations suggest that rot not only accompanies but also is one of the contributing factors to early senescence. Any program for increasing the length of the bearing life of apple trees in this region will have to take this into account.

It seems worth while to emphasize the well-known fact that most rot in these trees enters through wounds of one kind or another. If it were prac-

tical to prevent or reduce the incidence of such wounds, it is probable that decay might at least be delayed and the productive life of apple trees in this and similar regions increased considerably. Pruning branches when small, covering all pruning and other wounds, no matter how small, with a fungicidal wound dressing, bracing the larger branches to prevent splitting, and painting or wrapping the trunks to prevent sunscald might yield a higher return in what may be termed the marginal apple-growing regions, such as Minnesota, than in localities where the environment approaches the optimum for the growth of these trees.

SUMMARY

Decay of limited extent was found in 48 of 50 trees 15 years old at 3 feet above the ground. Decay extended throughout most of the diameter of the trunk of 12 of 68 trees 22 years old. Moderate to slight decay was found in 44 of the remaining 56 trees. Most trees 30 or more years old were thoroughly decayed, rot often extending out to or through the bark over part of the circumference of the trunk, appearing in some cases to have weakened or killed the cambium and inner bark.

Branch stubs were the chief means of entry of the rot into the tree. Other avenues of entrance were crotch cracks, sunscald injuries, and frost cracks.

The spread of rot within the tree was facilitated by sunscald, cold injury (blackheart), frost cracks and other wounds.

Ten species of wood-rotting fungi associated with this decay were identified. An equal or probably even greater number of species of wood-rotting fungi were isolated but could not be identified. No one species or combination of species was found frequently enough to be considered responsible for the major part of the decay.

The opinion is expressed that rot not only accompanies, but also may be one of the causes of, the early decline of apple trees in marginal regions of apple production. It is suggested that the prevention and proper care of wounds may increase the bearing life of these trees.

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RESISTANCE OF POTATO TO VIRUSES A AND X, COMPONENTS OF MILD MOSAIC

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In potato-producing localities like Northern Maine, mild mosaic is one of the primary limiting factors in the production of certified seed potatoes of certain varieties. This disease caused by the two viruses, A + X, occurs widely on Green Mountain, Triumph, and Rural New Yorker, and, if every plant is affected, may cause a reduction in yield of 15 to 25 per cent. Inasmuch as these varieties generally carry virus X, they manifest the mild mosaic reaction after aphids infect them with virus A.

The reaction of Green Mountain and Triumph to virus A alone has not been observed because these varieties harbor virus X. The reaction of a virus-free Green Mountain seedling, which very closely resembles the parent variety, may, however, give a good idea of the effect of virus A alone on Green Mountain. Virus A on this seedling induces light-green and slightly rugose foliage, whereas virus X induces light-green foliage and viruses A + X cause a reaction like mild mosaic on Green Mountain. Moreover, the type of reaction is determined by the strain of virus X involved; in combination with virus A, the more virulent strains of virus X induce more marked modifications than the weaker strains of this virus (4). The more pronounced symptoms may involve crinkling of the foliage.

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The virus A component was classified as virus A by Murphy and McKay (1) and as Solanum virus 3 by Smith (8). The virus X component was classified as virus X by Smith (7), and as Solanum virus 1 by Smith (8). This paper records information on the reactions of potato seedling varieties and the breeding behavior of their parents to virus A and to viruses A + X.

METHODS USED IN TESTING PLANTS FOR RESISTANCE

As in investigations previously recorded (3), 10 hills of each variety were planted in rows adjacent to rows of mild-mosaic Green Mountains in the first year field-exposure test. Two tubers per hill were harvested for planting as whole tubers, making 20 hills per seedling for field exposure to mild-mosaic Green Mountain in the second year. Only 1 tuber per hill was reserved for field exposure tests after the first year. This procedure provided for detection of plants infected too late for current-season symptoms to be expressed and also had the advantage of again exposing healthy plants to infection.

Inasmuch as it is frequently impossible to make an authentic diagnosis of the virus involved from the foliage reaction of the seedlings, the tubers from mosaic hills were reserved for use in graft inoculation on Green Mountain. Moreover, tubers from varieties that showed no disease were saved for inoculation on Green Mountain to determine if any of these were carriers of the virus. For detections of virus X, juice inoculations were made on *Datura stramonium* and *Capsicum* sp. For diagnosis of virus A, these varieties were inarch grafted on Green Mountain.

Inarch grafting, as previously described (2, 6), was used for testing the reaction of varieties that failed to contract virus A in the field, to ascertain whether such varieties also were resistant to or possibly immune from virus A in grafts.

It was recorded previously (5) that 14 per cent of the seedling varieties of No Blight × Katahdin contracted virus A in a field exposure test in 1934 on Aroostook Farm, Presque Isle, Maine. These seedling varieties were again exposed to mild-mosaic Green Mountain in 1936 and 1937. Unusually favorable conditions for field infection of virus A prevailed during 1937; infection was so general that every hill in most of the Green Mountain controls contracted virus A and none of the controls of this variety escaped infection.

REACTION OF POTATO SEEDLING VARIETIES TO VIRUS A

The results obtained from the seedling varieties No Blight × Katahdin (Table 1) disclose that of the 347 seedlings 47 per cent contracted virus A; every one of the Green Mountain and No Blight controls became infected but none of the Katahdins. Some of the seedlings reacted to virus A by faint mottling, and others by distinct mottling. Many of these seedling varieties contracted virus X in addition to virus A and this composite infection was manifested by a yellow mottling.

TABLE 1.—*Resistance to Virus A, a component of mild mosaic, as shown by the progenies of (No Blight × Katahdin), (Katahdin × Earlaine), and (Russet Rural × S 24642) in field exposure to mild-mosaic Green Mountain*

Pedigree or variety	Number of seasons exposed	Total number varieties or plots	Virus-A infected varieties or plots		Virus-A hills in infected varieties
			Number	Per cent	Average per cent
No Blight × Katahdin	3	347	162	47	25
Green Mountain	1	26 ^a	26	100	94
Katahdin	2	12 ^a	0	0	
No Blight	2	13 ^a	13	100	66
Katahdin × Earlaine	3	111	16	14	38
Green Mountain	1	12 ^a	12	100	88
Katahdin	3	5 ^a	0	0	
Earlaine	3	5 ^a	0	0	
Russet Rural × S 24642	1	39	18	46	25
Green Mountain	1	3 ^a	3	100	98
Russet Rural	1	3 ^a	3	100	54
S 24642	1	3 ^a	0	0	

^a Number of 20-hill control plots.

Of the infected seedling varieties, more than 50 per cent had virus A in only a few hills, while every hill in many Green Mountain controls contracted virus A, which indicates that many of the infected seedling varieties are more resistant to virus A by aphid infection in the field than is Green Mountain. The data show that the resistance of the Katahdin parent, which is virus-A immune by aphid infection, is transmitted to many varieties in this cross. Furthermore, it is possible that some of this resistance is inherited from No Blight, which apparently contracted virus A less generally than Green Mountain.

Fifty-four of the seedling varieties that did not become infected in field exposure were inarch grafted to virus A seedling variety 41956 and Green Mountain. Every one of these contracted virus A, which indicates that many of the No Blight × Katahdin seedling varieties react to virus A like Katahdin, viz., are virus-A immune by aphid infection but are virus-A susceptible by graft infection.

Of the 111 varieties of Katahdin × Earlaine, 14 per cent became infected with virus A and manifested this infection by light-green, faintly mottled, distinctly mottled, and slightly rugose foliage. Every Green Mountain control became infected, while the Katahdin and Earlaine controls remained healthy.

Of the 39 seedling varieties of Russet Rural × S 24642, 46 per cent contracted virus A and manifested this infection by light-green, faintly mottled and crinkled leaves. Every Green Mountain and Russet Rural control became infected with virus A, while S 24642 remained free from it.

A comparison of the reaction of the progenies of Russet Rural × S 24642 with that of the varieties of No Blight × Katahdin shows that about the same percentage of varieties is virus-A susceptible by aphid infection. In these

crosses the Russet Rural and No Blight are virus-A susceptible, while the S 24642 and Katahdin are virus-A immune by aphid infection. If both parents are virus-A immune by aphid infection, as in Katahdin \times Earlane, the percentage of susceptibles in the progeny is much lower than where one of the parents is susceptible. The 14 per cent of virus-A susceptibles in Katahdin \times Earlane discloses that these varieties are not homozygous for resistance.

REACTION OF POTATO SEEDLING VARIETIES TO VIRUSES A AND X

Previous work (6, 9) has shown that 37 per cent of the seedling varieties of virus-X immune 41956 \times virus-X susceptible Katahdin are virus-X immune by graft infection. The progenies of 2 such crosses and of another cross represented by S 41956 \times virus-X susceptible Earlane were exposed to mild-mosaic Green Mountain in the field. Inasmuch as Katahdin and Earlane are virus-A immune by aphid infection, these crosses should result in some progenies having resistance to viruses A and X.

The reactions of the above progenies to these viruses (Table 2) disclose that of the 76 seedling varieties of S 41956 \times Katahdin, 18 per cent contracted virus A and 30 per cent became infected with virus X. Of the 127 varieties of a second group of S 41956 \times Katahdin seedlings, 26 and 34 per cent contracted viruses A and X, respectively. Of the 136 seedling varieties of S 41956 \times Earlane, 23 and 62 per cent became infected with viruses A and X, respectively. All of the Green Mountain, Green Mountain seedling, and S 41956 controls contracted virus A, one Katahdin control, and every one of the Green Mountain seedling and Earlane controls contracted virus X, while S 41956 remained free from this virus.

The mosaic reaction of S 41956 to virus A, viz., light-green, faintly and diffusely mottled foliage, was manifested by most of the virus-A infected seedlings. The symptoms of virus-X-susceptible Earlane, light-green and faintly mottled foliage, were manifested in most of the virus-X-susceptible varieties of S 41956 \times Earlane.

The higher percentage of virus X seedling varieties from the S 41956 \times Earlane cross than from the S 41956 \times Katahdin cross appears to be related to the difference between Earlane and Katahdin in resistance to virus X infection; in the field Earlane is more easily infected with this virus than Katahdin.

IMMUNITY OF SEEDLING VARIETIES FROM VIRUSES A AND X AS SHOWN BY GRAFTS

Thirty-seven virus-X immune varieties of the cross S 41956 \times Earlane found to be virus-X immune in grafts (6, 9), were grafted on virus-A infected seedling variety 41956 to determine whether any of them were immune from virus A. The result of these grafts disclosed that of the 37 virus-X immune varieties, 14 developed apical or top necrosis from virus A, while the remaining 23 varieties manifested light green and faintly mottled leaves as a result of virus-A infection. Inasmuch as Earlane develops top

necrosis in grafts to virus-A-infected varieties but is virus-A immune by aphid infection, it is reasonable to assume that the 14 virus-X-immune varieties by graft infection are also virus-A immune by aphid infection.

DISCUSSION

The work involved in testing potato varieties for resistance to viroses is complicated by differences between varieties in their reaction to a single virus, by the similarity of symptoms frequently produced by different viruses, by the existence of virus strains, and by variations in aphid infestations in the field.

Even after field-exposure tests, graft and juice inoculations from the exposed varieties must be made to key varieties in order to identify the virus involved. Unless the tests are conducted under favorable aphid infestations, as was done in 1937, it is necessary to expose the varieties in the field more than one season to obtain reliable results.

Certain varieties were completely infected with virus A when numerous aphids were used under cloth cages, while under average field conditions less than 10 per cent of the hills in these varieties contracted this virus. Furthermore, as has been shown, certain varieties are susceptible to virus A by graft inoculation, but immune from it by aphid inoculation. It is apparent that virus infection by graft inoculation does not necessarily indicate that such varieties are susceptible to the same virus by aphid inoculation.

The self-fertile varieties Earlane, Katahdin, and S 24642, used as parents in studies on virus-A resistance as recorded in this paper, are immune from virus A by aphid infection, but are susceptible to this virus by graft infection. Katahdin and S 24642 react to virus A in graft tests by faint mottling. Earlane reacts to this virus in graft tests by top necrosis. Virus A can be recovered from the tubers of graft-inoculated Katahdin and S 24642, but it has not been recovered from the tubers of the graft-infected Earlane, which suggests that virus-A may be inactivated in the necrotic areas of Earlane.

Earlaine, Katahdin, and S 24642 are genotypically similar in their reaction to virus A. These varieties are not homozygous for resistance to this virus, as shown by tests of progenies from selfed Katahdin and from the Katahdin \times Earlane cross. The crosses involving virus-A susceptible No Blight \times virus-A-immune Katahdin and virus A-susceptible Russet Rural \times virus A-immune S 24642 produced 53 and 54 per cent virus-A-resistant varieties by aphid infection, which indicates that Katahdin and S 24642 segregate for resistance to virus A.

S 41956 \times Earlane exposed for two seasons gave about the same results with virus A as the progeny S 41956 \times Katahdin tested during the same time. The X^2 for independence is less than 1. This indicates that Katahdin and Earlane are genotypically alike with respect to resistance to this virus.

Virus-A-immune varieties by aphid infection that also are immune from this virus by graft infection have not been found in these progenies. How-

ever, the necrotic reaction involving tuber and top necrosis, as manifested by Earlane in graft infections with virus A, appears to indicate a resistant host reaction to this virus. Inarch grafts involving stalk grafts of virus-A Green Mountain to healthy Earlane to virus-A-free Green Mountain conduct virus A to Green Mountain, which indicates that Earlane conducts this virus. Virus A, however, has not been recovered from necrotic tubers of virus-A-infected Earlane.

Many of the virus-A-susceptible seedling varieties contracted this virus in less than 10 per cent of the hills during 3 seasons in the field, whereas over 90 per cent of the Green Mountain hills became infected during one season. This indicates that these virus-A-susceptible varieties contract this virus less easily than varieties like Green Mountain under field conditions. That a higher percentage of infected hills in such virus-A-susceptible varieties can be obtained with very heavy aphid infestations under cloth cages shows that aphid dosage, in part at least, may affect these results.

Although both Earlane and Katahdin contract virus X in the field, Earlane becomes infected much more easily with this virus than Katahdin. In the field-exposure tests it was found that 62 per cent of the varieties of S 41956 \times Earlane contracted virus X, while 34 per cent of the varieties of S 41956 \times Katahdin became infected with this virus. Furthermore, the average percentage of the virus-X-infected hills was 20 per cent more in the progenies of the former than in those of the latter cross. As previously shown, S 41956 is immune from virus X.

Inasmuch as 66 per cent of the progeny of S 41956 \times Katahdin did not contract virus X in the field, and 37 per cent of these seedlings are virus-X immune in grafts, it is apparent that 29 per cent virus-X susceptibles in grafts rarely contract this virus in the field. This indicates that these susceptibles resemble Katahdin in virus-X resistance in the field.

SUMMARY

Experience with the reaction of potato seedling varieties to virus X has shown that on the basis of symptomatology varieties may be classified as (a) symptomless carriers, (b) necrotic, (c) light green and slightly rugose, (d) faintly mottled. On the basis of resistance to virus X, varieties may be grouped as (a) immune, (b) rarely infected, (c) easily infected.

Experience with the reaction of potato seedling varieties to virus A has shown that on the basis of symptomatology varieties may be classified as (a) necrotic, (b) light-green and rugose, (c) mottled. On the basis of resistance to virus A, varieties may be grouped as (a) immune, (b) rarely infected, (c) easily infected. Varieties are virus-A immune by aphid infection, but not by graft infection. It is indicated that the necrotic reaction of some varieties to virus A by graft infection suggests that such varieties are virus-A immune by aphid infection.

The resistance reaction of the parents to virus A or to virus X is transmitted to a high percentage of the progeny. A higher percentage of virus-A

or virus-X resistant progeny results from resistant \times resistant than from non-resistant \times resistant parents.

Earlaine, Katahdin and S 24642 are virus-A immune by aphid infection, but are virus-A susceptible by graft infection. These varieties apparently segregate for resistance and susceptibility to virus A in crosses with non-resistant varieties.

Progenies of virus-X immune \times virus-A immune have been produced that are immune from both viruses. Inasmuch as these 2 potato-mosaic viruses and their strains are among the 3 potato-mosaic-virus groups that are responsible for most of the potato mosaic epiphytotics, varieties immune from both virus A and virus X will play an effective rôle in control of potato mosaic.

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A SEXUAL PHENOMENON EXHIBITED BY CERTAIN ISOLATES OF PHYTOPHTHORA CAPSICI¹

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INTRODUCTION

In 1932, through the courtesy of George F. Weber of the Florida Agricultural Experiment Station, the junior writers received a culture of *Phytoph-*

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² The writers wish to express their appreciation to Dr. C. M. Tucker of the Department of Botany of the University of Missouri for verification of the identity of the isolates used in this study.

thora capsici Leonian, which, when grown in the same culture dish with any one of several Colorado isolates of the same organism obtained from blighted pepper plants (*Capsicum annuum* L.), showed oospore production in the zone of the junction of the two converging cultures. The experiment was repeated many times on different types of media, with the same result. Pairing in the same way any two Colorado isolates from peppers failed to show the formation of oospores.

Although Weber (6) reported oospore formation in his isolates, none were produced by the culture he sent. In like manner no oospores were produced by any of the Colorado isolates when grown on oatmeal, cornmeal, and hard potato-dextrose agars for 3 months at a time. It is more or less agreed that isolates of *Phytophthora capsici*, the causal agent of pepper blight, will readily produce oospores in culture (2, 4, 6). However, in a written communication in 1936, C. M. Tucker stated that he had found considerable variation in cultures of *P. capsici* with regard to oospore production, "some producing oogonia quickly, others after a long period only, and some not at all." Moreover, Tompkins and Tucker (5) reported in 1937 that 10 of the 13 cultures of *P. capsici* they obtained from Honeydew melon (*Cucumis melo* L. var. *inodorus* Naud.) produced oospores only after having been kept in culture for 4 months.

EXPERIMENTAL

Source of Cultures

The cultures used in this study were isolated from cucumber fruits (*Cucumis sativus* L.) that were decaying in the field and from the stems of wilted pepper plants. Isolate 26A was obtained from diseased cucumber fruits in a field near Rocky Ford, Colorado, in August, 1936 (1). In August, 1937, 3 more cultures of the organism were isolated. Isolate 59A was obtained from the stems of dying pepper plants near Pueblo, Colorado, isolate 60A from stems of wilted pepper plants in the Rocky Ford region, and isolate 61A from rotting cucumber fruits in a field near Florence, Colorado. The isolates from cucumber fruits were found as pathogenic to uninjured 6- to 9-week-old pepper plants (varieties Italian Bell, California Wonder, and Chinese Giant) as those obtained from the stems of pepper plants showing the characteristic blight. After having been tentatively identified as isolates of *Phytophthora capsici*, the cultures were submitted to C. M. Tucker of the University of Missouri, who verified the determinations.³

Oospore Production

As stated previously, no culture of *Phytophthora capsici* had been observed to produce oospores, though many of these isolates had been grown on the various types of standard laboratory media for several months at a time. In addition, as mentioned earlier, oospore production had been observed only as a consequence of pairing a Florida isolate with a Colorado

³ See footnote 2.

isolate. Because of the results of this earlier work, the recently isolated cultures of *P. capsici* were paired in all possible combinations on oatmeal agar, potato-dextrose agar, and Tucker's corn-meal agar. The results of this study are given in table 1.

TABLE 1.—Oospore production in nutrient agar media as a result of pairing cultures of *Phytophthora capsici*

Culture pairings	Oospore production		
	Potato-dextrose agar	Corn-meal agar	Oatmeal agar
26A (cucumber)—59A (pepper)	+	+	+
26A (cucumber)—60A (pepper)	+	+	+
26A (cucumber)—61A (cucumber)	—	—	—
59A (pepper)—60A (pepper)	—	—	—
59A (pepper)—61A (cucumber)	—	+	+
60A (pepper)—61A (cucumber)	+	+	+

It will be observed that oospore formation occurred only when both the pepper and cucumber isolates were grown in the same culture dish, pepper-pepper and cucumber-cucumber isolate pairings giving negative results.

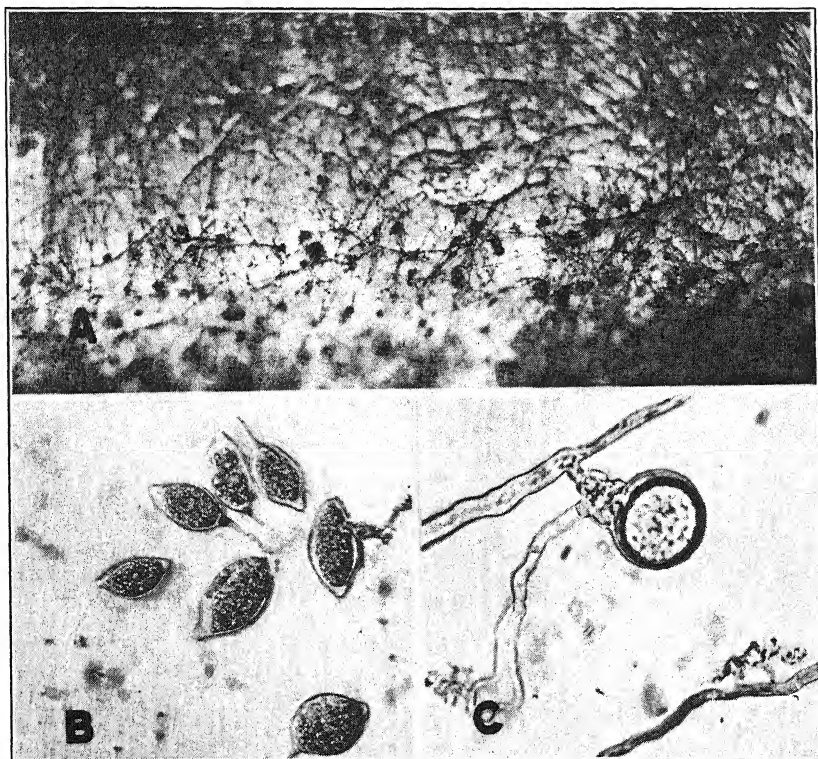


FIG. 1. *Phytophthora capsici*. A. Oospores at the juncture of two paired cultures of *P. capsici* (isolates 59A-26A). B. Sporangia of *P. capsici* (isolate 60A). C. Sexual apparatus of *P. capsici*, showing the antheridium and oogonium.

In the pairings 26A-59A and 26A-60A, oospores were produced in abundance, while in the 59A-61A and 60A-61A pairings, oospores were not so abundant. In all instances where oospores were produced, their occurrence was at the junction of the mycelia of the two cultures (Fig. 1, A). The formation of these bodies was observed within 24 hours after the hyphal tips of the converging cultures met. The failure of the 59A-61A combination to produce oospores on potato-dextrose agar may be evidence of a nutritive influence on the factors governing the formation of these bodies. This study was repeated several times, giving in each case essentially the same results as those just described.

In order to ascertain the origin of the sexual organs produced at the mycelial junction of paired cucumber and pepper cultures, attempts were made to trace antheridial and oogonial hyphae to their source thalli. To obtain a sparse growth and still obtain oogonia, paired isolates were grown on cleared corn-meal agar ranging from 0.5 to 2 per cent of nutritive material. Oogonia and amphigynous antheridia appeared quite distinctly in the majority of the fields examined (Fig. 1, C). The hyphae producing oogonia were in no case observed to give rise to antheridia, and those mycelia to which the antheridial hyphae could be traced never showed any direct connection with an oogonium. In spite of this observation, however, male and female hyphae could not be traced to their source thalli with any degree of certainty.

Tests involving the use of vital dyes (1 per cent aqueous solutions of cotton blue, neutral red, and methylene blue) in attempting to stain differentially the mycelia of one of the two cultures in any plate resulted in failure, due to the slow diffusion of any dye used. Narasimhan's mica-strip technique (3) gave no better results, since, by the time recognizable sexual bodies were formed, hyphal interlacing was already too great to allow tracing with accuracy.

Because of the findings of Tompkins and Tucker (5) relative to oospore production by *Phytophthora capsici*, the cultures under investigation were transferred to 500-ml. Erlenmeyer flasks containing Tucker's differential media (4), i.e., potato-dextrose agar, oatmeal agar, corn-meal agar, bean-meal agar, steamed corn meal, and steamed bean pods. These cultures, held

TABLE 2.—Oospore production by isolates of *Phytophthora capsici* after having been grown for eight months on Tucker's differential media

Isolate number	Oospore production					
	Corn meal	Corn-meal agar	Bean-meal agar	Bean pods	Potato-dextrose agar	Oat-meal agar
26A	—	—	—	+	—	—
59A	—	—	—	—	—	—
60A	—	—	—	—	+	—
61A	—	—	—	+	(sparse)	—

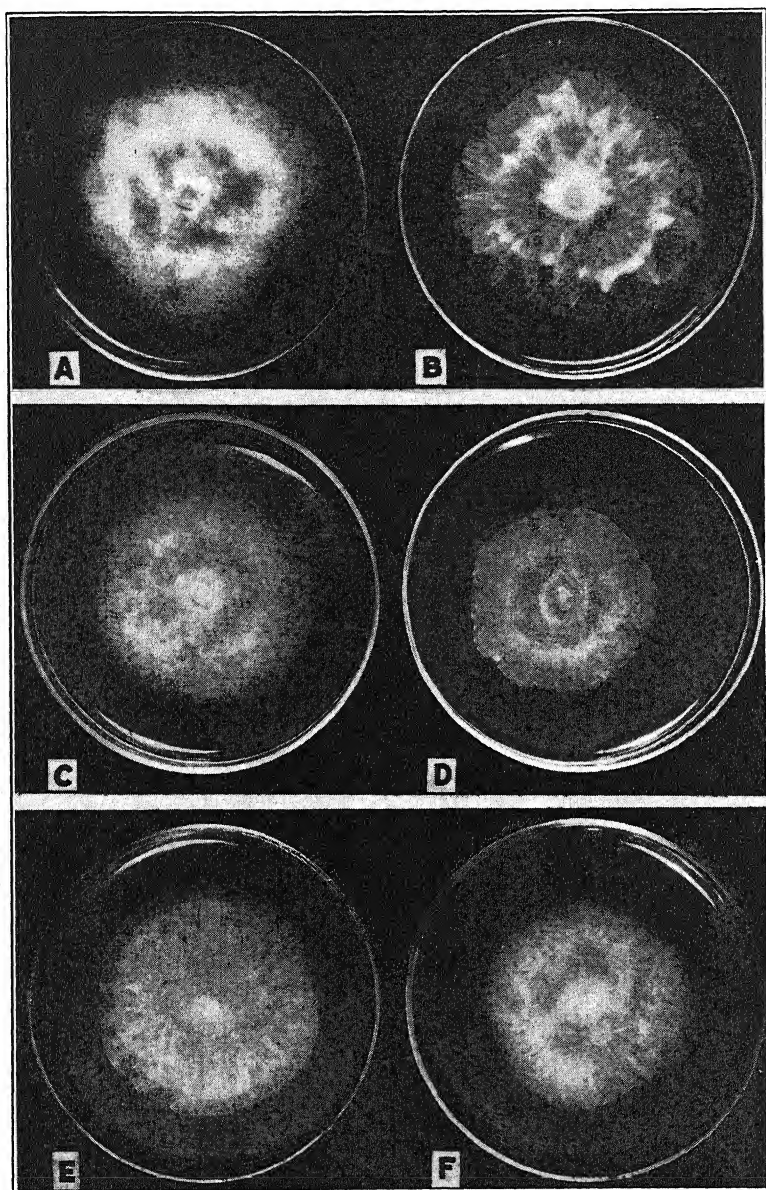


FIG. 2. Isolates of *Phytophthora capsici*. A. Pepper isolate (Florida, 1932). B. Pepper isolate (Rocky Ford, Colo., 1931). C. Pepper isolate No. 59A (Pueblo, Colo., 1937). D. Pepper isolate No. 60A (Florence, Colo., 1937). E. Cucumber isolate No. 26A (Rocky Ford, Colo., 1936). F. Cucumber isolate No. 61A (Florence, Colo., 1937).

at room temperature, were examined for the presence of oospores at monthly intervals for 8 months, when final readings were taken. The results of this study are shown in table 2.

Oospores were first observed in cultures of 26A and 61A (cucumber fruit

isolates) growing in flasks of steamed bean pods at the end of 6 months. Oospores were not produced by these isolates in the other media employed. Cultures of 60A (pepper isolate) showed these bodies at the end of the 8-month period on potato-dextrose agar only. Cultures 26A and 61A produced abundant oospores, whereas cultures of 60A showed but few. Cultures of 59A produced no oospores in any of the media used, even after remaining in culture for 12 months.

Certain Morphological and Physiological Comparisons of the Isolates of *Phytophthora capsici*

The cultures of *Phytophthora capsici* studied showed no constant morphological differences. Whereas the majority of the earlier isolates obtained from Colorado material (1931-1933) had shown a characteristic starred effect when grown in culture (Fig. 2, B), few of the later isolates showed this cultural manifestation (Fig. 2, C, D, E, and F). It will be noted in figure 2, A, that the Florida isolate did not show this type of growth in culture.

Sporangia of all isolates were produced in abundance when uniform pieces of fresh cucumber or squash tissue were transferred to sterile water blanks, inoculated, and incubated at 20, 25, 30, and 35 degrees C. for 48 hours (Fig. 1, B). On the tissue of green pepper fruits, isolates 26A, 60A, and 61A produced very few sporangia at these temperatures, whereas isolate 59A produced none. The optimum temperature for sporangial production for all isolates studied appeared to be near 30° C. No sporangia were produced by any isolate at 15° C. Reducing the size of the piece of tissue appeared to accelerate the formation of sporangia.

After oogonia and oospores were found in the 6- to 8-month-old cultures, a comparison of the sizes of these bodies was possible. Accordingly, as shown in table 3, a series of measurements was made, each average given representing a total of 100 spore measurements.

TABLE 3.—Comparison of measurements of oogonia and oospores of isolates 26A, 61A, and 60A

Culture number	Ave. diameter oospores (μ)	Ave. diameter oogonia (μ)	Extremes of measurements (μ)
26A	27.96	31.95	19.5-22.5 to 36.0 -42.0
61A	26.94	31.32	19.5-24.0 to 41.25-45.0
60A	30.54	34.59	18.0-22.5 to 39.0 -45.0

It may be observed in table 3 that the cucumber isolates (26A and 61A), which were grown on bean pods, showed only slight differences between their average spore measurements. Apparently larger spores were produced by the pepper isolate (60A), which was grown on potato-dextrose agar.

SUMMARY

Pairing in the same Petri dish a culture of *Phytophthora capsici* obtained from the stem of a wilted pepper plant with one isolated from decaying

cucumber fruit resulted in the formation of oospores. These bodies were formed in the zone of the junction of the two converging cultures 24 hours after their hyphae met. No such phenomenon was observed when pepper isolates were paired or when cucumber isolates were paired. In the case of any cucumber-pepper isolate pairing, attempts to trace the antheridial and oogonial hyphae to their source thalli resulted in failure.

Only 3 of the 4 cultures studied produced oospores when grown separately. In such cases oospores were formed only after the isolates had been held in culture for from 6 to 8 months. These bodies were produced by 1 pepper isolate on potato-dextrose agar only. Oospores were formed by the cucumber isolates on bean pods only. A comparison of the sizes of these structures was made. In addition, observations were made regarding the influence of certain factors on sporangial production by the different isolates studied.

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OBSERVATIONS ON POLYPORUS CIRCINATUS¹

CLYDE M. CHRISTENSEN

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Polyporus circinatus Fries infects and decays the roots and lower part of the trunk of several species of conifers, causing a white pocket rot that was first accurately described by Hubert (2). Publications by Faull (1), Hubert (2), Lowe (3), and Solovieff (4) indicate that the fungus occurs throughout the coniferous forests of northern United States, southeastern Canada, and Russia, and that it sometimes causes considerable loss of timber, especially in predisposing trees to windthrow.

At Itasca Park, in northwestern Minnesota, the author has found *Polyporus circinatus* commonly on white spruce (*Picea glauca* (Moench) Voss), occasionally on black spruce (*P. mariana* (Miller) Br. St. and Pog.), Jack pine (*Pinus banksiana* Lambert), and balsam fir (*Abies balsamea* (Linnæus) Miller), and once on red pine (*P. resinosa* Aiton).

The range in variation in macroscopic and microscopic characters among nearly 100 fruit bodies of *Polyporus circinatus*, found in or near Itasca Park over a period of 10 years, exceeded only slightly that given by Lowe (3).

¹ Paper No. 1792 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

Microscopically, the fungus is characterized by brown, pointed setae that arise from colored hyphae in the pore wall and project out into the pore (Fig. 1). Using an eyepiece micrometer and an oil-immersion lens, the

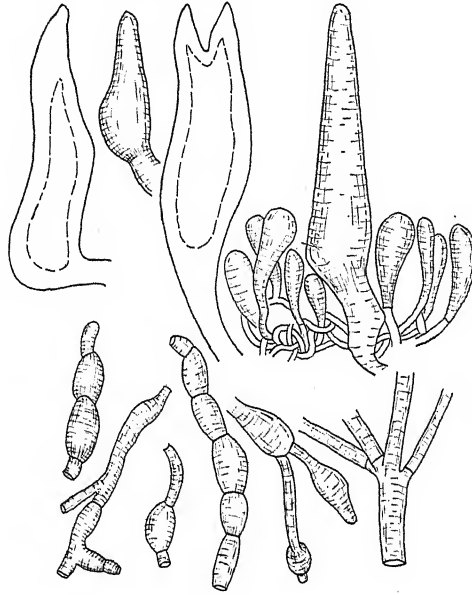


FIG. 1. Upper row, setae of *Polyporus circinatus*, the one at the right with other elements of the hymenium. Lower row, chlamydospore-like cells from cultures of *P. circinatus*, and, at the right, the method of branching found commonly in cultures. $\times 710$.

writer measured 20 setae from each of fruit bodies and 40 from a seventh. The average length of the 160 setae was $44\ \mu$, the range from 23 to $79\ \mu$. The lowest average length of setae from one fruit body was $39\ \mu$ and the highest average from a single fruit body was $57\ \mu$, which indicates fairly well the range of variation. Two double-pointed setae were found among the several thousand observed. The spores are hyaline and approximately oval, with an apiculus evident under oil immersion. Using a screw micrometer and an oil-immersion lens, 150 spores were measured, 50 from each of 3 fruit bodies. These were mounted as follows: A drop of water containing spores was placed on a slide, a small drop of melted agar, cooled almost to the point of solidification, added to it, and a cover slip placed over this and quickly pressed down. Previous studies on the measurement of small spores had indicated that this method did not alter the apparent size of spores (when compared with spores mounted in distilled water alone), as some mounting fluids and even vital stains do, and it had the virtue of holding the spores quite still. The spores from the 3 fruit bodies averaged 4.7 , 4.7 , and $5.2\ \mu$ in length, respectively.

At Itasca Park, in 1937, several groups of 10 to 50 trees each were found in which up to 50 per cent of the living trees bore fruit bodies of the fungus. Some fruit bodies also were found on the roots of dead trees, particularly

black spruce, and these trees also tended to be in groups. During the 2 years preceding and the 2 years following 1937 only a few scattered fruit bodies were found in these areas, and none elsewhere. Although the observations were necessarily limited in time and area, they seemed to indicate occurrence of definite loci infection, but this has not been correlated with any environmental factors.

It has been stated that most of the damage caused by *Polyporus circinatus* is in predisposing trees to windthrow, but it is doubtful if this is entirely true. Judging from his own observations, the writer believes that most of the trees, windthrown because of decay by this fungus, could not have survived many years, even had they not been blown over. The evidence for this has been gathered from an examination of numerous infected living trees and from trees recently windthrown from rot caused by *P. circinatus*. One typical example will be described. In 1937, 3 fruit bodies of *P. circinatus* were found on the roots of a codominant white spruce about 12 in. in diameter at stump height and 50 years old, growing on a fairly good upland site. The only outward symptom of infection, other than the presence of fruit bodies, was a certain unthriftness of the tree—the needles were shorter and less numerous than on healthy trees. This tree was felled, the roots grubbed out for a distance of about 4 feet from the stump, and dis-

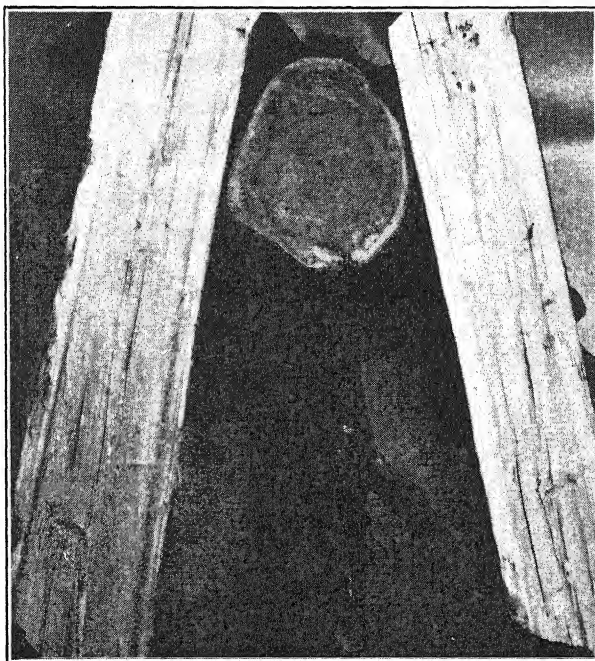


FIG. 2. Stump of tree decayed by *Polyporus circinatus*. Note the discoloration on the surface of the stump extending out almost to the cambium. The longitudinal sections left and right were the first and second sections, respectively, above the stump, each of them about $3\frac{1}{2}$ feet long. Visible decay extended 6 feet above the stump.

sected. Infection apparently had begun on one of the lateral roots about 3 feet from the stump, had advanced from there to the root crown, out into the other main roots and up into the trunk of the tree. On the side of the original infection the wood of the root crown was in the final stages of decay, so friable that it could be crumbled in the hand, but outward on the other main roots and upward in the trunk the decay had not yet reached this stage. In all of the larger roots, however, and in the trunk of the tree at stump height (about 18 in. above the ground) the fungus had penetrated out to the cambium. The tree had almost ceased growing 12 years earlier, and the last 12 annual rings were visible only in microscopic section, each ring being from 3 to 15 cells wide. Sections from this tree are shown in figures 2 and 3. Obviously, this tree was about to die regardless of whether it stood up, fell over, or was blown over by the wind.

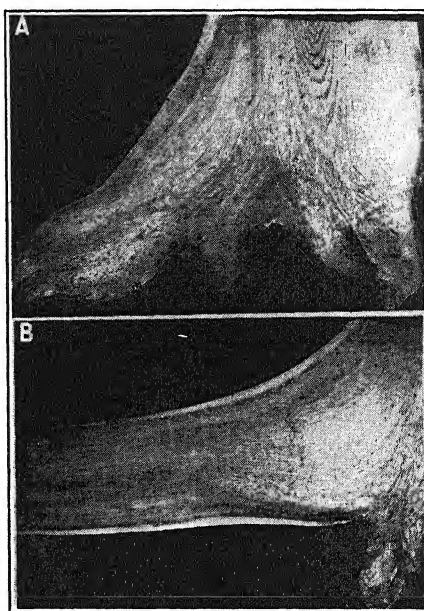


FIG. 3. A. Section through the center of the stump shown in figure 2. B. Section through one of the large lateral roots of the same stump. Note that infection seemed to be progressing from the stump to the distal end of the root. This root was on the side opposite the source of infection.

In August, 1938, about 30 newly windthrown white spruce from 6 to 10 in. in diameter were found scattered through the areas where fruit bodies had been common the year before. All of these apparently had been felled at the same time, by a heavy windstorm that was known to have occurred about a month before, and their leaves were still fresh and green. The roots and basal portion of the stem of all of these trees were rotted, the majority of them by *Polyporus circinatus*, although rot apparently due to *P. guttulatus* was found in a few. Several were dissected and cultures of *P. circinatus* obtained from various portions of the rot column. The decay in all

of these trees was essentially identical with the symptoms described in detail above. Judging from the dissection of these trees and the partial dissection of numerous others, the fungus often must infect the roots of trees no more than 15 to 25 years old, or even younger. Apparently it progresses rather slowly, but when decay extends out into or near the cambium of most of the larger roots and the lower part of the trunk, the tree ceases growing and eventually falls or is blown over. In other words, the fungus does not merely accompany stagnation and senescence but, if present at all, is likely to be one of the primary causes of such conditions. In this respect it resembles somewhat *P. schweinitzii* as described by York (5). If it does not seem abundant enough to occasion alarm at present, it still is sufficiently common to deserve some attention, and there always is the possibility that, like *P. schweinitzii*, it may become far more important in plantations than in natural forests. It is hoped that experiments now under way will answer some of the questions concerning mode of entrance of the fungus into the roots and the factors that influence infection.

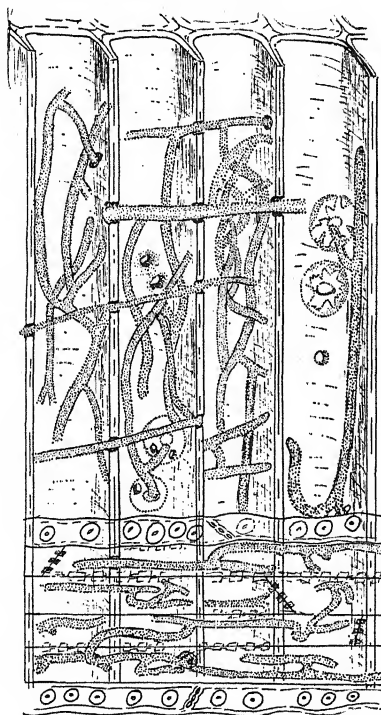


FIG. 4. Microscopic section through reddish zone at the periphery of decay at stump height, taken from the stump in figure 2. This shows a typical nest of mycelium, where a pocket probably would form later.

MICROSCOPIC CHARACTERS OF DECAY

Mycelium is rather sparse in the early stages of decay, where the wood is just faintly discolored, but nests or clumps of mycelium can be found at rather regular intervals some time before pockets are formed, especially in

the dark-reddish zone at the periphery of the rot column. The pockets are initiated most abundantly just at the junction of the annual rings, and, although the pockets in that region extend farther into the first few cells of the springwood than into the last few of the summerwood, they are not restricted to springwood. Because of the prevalence of pockets in this region, the wood in the early stages of decay tends to separate along the rings as it is dried. In the later stages of decay, however, when pockets are distributed uniformly through the wood, this tendency no longer is noticeable. The hyphae penetrate the walls of tracheids and parenchyma cells frequently and form rather large bore-holes. Essentially all of the wood elements are digested in the formation of pockets, and in the final stage of decay the wood forms only an interlacing network between the pockets, and eventually even this network is largely consumed. A variety of other microorganisms probably aid in the final decomposition, since they occur in abundance in the later stages of decay. The mycelium penetrates out to the cambium and apparently kills it. Considerable resin is exuded between the wood and the bark in regions where the fungus recently has grown out through the cambium, and this sometimes seeps out into the soil and hardens, so that hard clumps of earth infiltrated with resin are found around the infected roots. The microscopic characters of the early stage of decay are illustrated in figure 4.

CULTURAL CHARACTERS OF POLYPORUS CIRCINATUS

The fungus can be and repeatedly has been isolated fairly readily from sporophores and from all portions of the rotted wood except the advanced stage, where other organisms are likely to be present. Either malt agar or potato-dextrose agar is a suitable culture medium. The cultures grow slowly, transfers from fresh, actively growing colonies attaining a diameter of from $\frac{1}{4}$ to $\frac{3}{4}$ cm. in a week; and cultures of some isolates never attain a diameter of more than a few centimeters, even on agar in 250 cc. flasks. The color of the mycelium varies from light yellowish-brown to dark-chocolate, and the aerial mycelium forms a dense, firm, appressed mat. Hemispherical protuberances grow up at the center or near the margin of cultures of some isolates, resembling the abortive fruit bodies formed in culture by some wood-rotting fungi. The mycelium is without clamp connections, but both aerial and submerged hyphae of cultures 1 to 2 months old bear numerous chlamydospore-like enlargements, shown in figure 1. In this latter character, as well as in general cultural characters and type of rot caused, this fungus resembles *Fomes pini*, but cultures of *Polyporus circinatus* usually can be distinguished from those of *F. pini* by the faster growth and more abundant aerial mycelium of the latter, as previously stated by Hubert. The writer's cultures of *F. pini* (from white pine in Minnesota and sugar pine in California) could readily be distinguished from those of *P. circinatus* on those bases.

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THE CONSTRICTION DISEASE OF PEACH

JOHN W. ROBERTS

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INTRODUCTION

In April, 1938, F. P. Cullinan, senior pomologist at the U. S. Horticultural Station, Beltsville, Maryland, turned over to the writer some diseased peach twigs and branches that he had collected in Delaware. The lesions on the twigs and the fungus fruiting on them indicated that the malady was the constriction disease described by Selby (12) in 1898. Selby considered the pathogen to be identical with *Phoma persicae* Sacc., although his identification appears to have been based on Saccardo's description, rather than comparison with type material. He apparently performed no inoculation experiments.

The material collected by Cullinan was sent to John A. Stevenson, senior mycologist in charge of Mycological Collections, Bureau of Plant Industry, for an opinion concerning the identity of the fungus. He confirmed the writer's diagnosis and suggested that the fungus was not a typical *Phoma*, but that the structure of the pycnidium indicated that it was probably a *Phomopsis*, although he could find no β conidia. He also reported that there is in the Collections a dried culture received from C. R. Orton, then of Pennsylvania State College, September 12, 1915, to which Anna E. Jenkins had attached the following note: "In culture this fungus has characters of *Phomopsis*. Specimen from which fungus was isolated not seen. This is evidently, however, the fungus described in Saccardo as *Phoma persicae*." Selby's (12) figure 3 of plate IX is a drawing of a plurilocular pycnidium characteristic of the genus *Phomopsis*. As will be shown later, the fungus is a *Phomopsis* and produces both α and β conidia in artificial culture. Stevenson also reported collections by S. A. Wingard in Virginia and B. B. Higgins in Georgia. The disease also is mentioned by Adams (1) as occurring in Delaware, Clinton (4) in Connecticut, Hesler and Whetzel (7) in New York, and Verwoerd and Du Plessis (13) in South Africa. The writer has also received specimens purported to have been collected in New Jersey. In company with K. J. Kadow, of the University of Delaware, the writer

visited the orchard in which F. P. Cullinan had collected his diseased material and studied the disease as it occurs naturally.

A disease of peach and other drupaceous fruits, known as "die-back," has been reported from Europe (2, 3, 4). This disease, or at least the canker phase of it, is apparently identical with the constriction disease and, as will be shown later, appears to be caused by the same fungus.

Since only brief descriptions of the disease as it occurs in the United States and no studies of the pathogen and suspect-pathogen relations have been published, it seems worth while to the writer to give a complete description of the disease, together with a report of his investigations of the pathogen and suspect-pathogen relationship.

DESCRIPTION OF THE DISEASE

Selby (12) showed rare judgment in naming this disease. The girdling or constriction of the branch by the fungus early in the growing season causes a wilting of the leaves and subsequent death of the branch above the constriction. The invaded areas or cankers are light-tan to brown, and

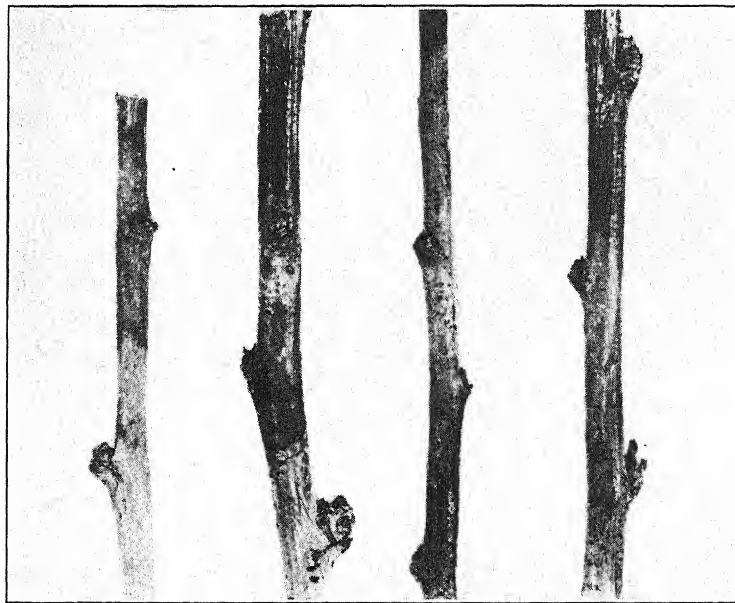


FIG. 1. Constriction disease of peach, showing cankers about dead buds.

usually are centered about a dead bud (Fig. 1). Evidently the fungus enters either through a dead bud or through dead tissues about the bud such as commonly result from injury by arsenical and sometimes by copper sprays. In the Delaware orchard only old, devitalized or injured trees were affected; adjacent trees in a high state of vigor were not attacked, although the cankered areas on the affected trees were producing immense quantities of conidia exuding from the numerous pycnidia either as cream-color masses

or as long cirrhi. Pycnidia are usually present on the lesions and, if not exuding masses of conidia, can be induced to do so by placing affected branches in a moist chamber for a day or two. No β conidia have been found in these conidial masses; but, since they may be produced in culture, they probably occur in nature. As is usual with peach cankers, gum production may be extensive.

Although affected trees often are severely injured, the disease must be considered a minor one that attacks weakened trees only. It seems to have attracted little attention, since Selby (12) described and named it in 1898.

PHOMOPSIS ISOLATES FROM TWIGGS OF PEACH AND OTHER FRUITS

The pathogen was isolated by means of a conidia, using the poured-plate method. In addition to 3 isolations from peach cankers, isolations of *Phomopsis* from other fruits were made for purposes of comparison. The following is a brief description of these isolates:

Peach I. Isolated from constriction disease of peach, Delaware. Growth rapid on oatmeal agar, white, flocculent, rarely producing pycnidia in slants, more freely in plates. Pycnidia produced once after 2 months on Mix (10) K. U. medium, but not subsequently. Beta conidia rare. Alpha conidia $8-9 \times 3-4 \mu$, faintly biguttate, but more often nonguttate, or faintly uniguttate. Beta conidia $28-36 \times 1-2 \mu$.

Peach II. Isolated from constriction disease of peach, New Jersey (?). Growth on oatmeal agar at first white, flocculated, later turning dark with the production of many pycnidia in both slants and plates. No β conidia found. Alpha conidia $8-10 \times 3-5 \mu$, often nonguttate or only faintly guttate. Differs from Peach I chiefly in more copious production of pycnidia.

Peach III. Isolated from constriction disease of peach, Delaware. Material collected from the same group of trees as Peach I, but 1 year later. Differs from Peach I and Peach II in that it quickly turns dark and produces pycnidia much more readily in cultures and in larger numbers, with more copious spore production and strongly guttate α conidia. One culture on oatmeal produced conidia copiously in 17 days with about 1 β conidium to 100 α conidia. Alpha conidia $6-8 \times 3 \mu$, distinctly biguttate. Beta conidia $26-33 \times 1-2 \mu$.

Sweet cherry. Isolated from a dead branch collected by the writer at Beltsville, Md. Resembles the peach isolates, but fruits much more readily than Peach I and II on cornmeal agar and oatmeal agar. Fruits on oatmeal agar and cornmeal agar in 12 days. Beta conidia abundant, becoming less so with age. Alpha conidia $8-10 \times 3 \mu$, distinctly biguttate. Beta conidia $24-36 \times 1 \mu$.

Apple. Isolated from a *Phomopsis* on a dead apple branch collected by the writer at Beltsville, Md. Growth on artificial media similar to that of peach isolates. Fruited readily on oatmeal agar in 12 days. Alpha conidia $8-10 \times 3-4 \mu$, distinctly biguttate. Beta conidia $24-32 \times 1 \mu$.

Pear. Isolated from a *Phomopsis* on a dead branch of pear collected at Beltsville, Md. Growth on artificial media resembles that of the peach isolates, possibly slightly darker. Pycnidia are readily produced on cornmeal agar and oatmeal agar. Alpha conidia $8-10 \times 3-4 \mu$, distinctly biguttate. Beta conidia $22-32 \times 1 \mu$.

All these isolates resemble one another rather closely. The chief difference is in the readiness with which they fruit on artificial media, and in this, Peach III resembles the pear, apple, and sweet-cherry isolates more closely than it resembles Peach I.

INOCULATION EXPERIMENTS

Since no one in the United States seems to have reported inoculation experiments with the constriction disease pathogen, an inoculation experiment on limbs of a peach tree growing out of doors was performed to determine

whether or not the fungus could enter (a) noninjured areas about buds, (b) areas about buds burned by a hot metal disk. Inoculations through the burned areas were successful, the fungus increasing the diameter of the spots from the original 4 mm. to as much as 18 mm. in 12 days. To prevent introducing a possibly new disease in the orchards, the twigs were removed before the fruiting bodies appeared on the surface. After 48 hours in moist chambers, pycnidia producing typical α conidia appeared in the killed areas. Neither the controls nor the noninjured but inoculated areas about the buds developed the disease.

In April, 1939, a series of inoculations on peach and apple trees kept in a humidified chamber in a greenhouse were performed to discover the comparative pathogenicity of all the forms of *Phomopsis* isolated from fruits, except Peach III, which had not yet been isolated. In these tests inoculations were through slits that were later covered with wet cotton. All isolates were able to infect peach branches through the wounds, but the peach isolates, particularly Peach II, were more dependable, and enlarged the spots and girdled the twigs more rapidly. Peach I was not so successful as Peach II, possibly because stromatic tissue rather than conidia had to be used as inoculum. Lesions caused by the peach, cherry, and apple isolates produced pycnidia and α conidia abundantly. All isolates were about equally successful in invading apple tissues through the slits. In 20 days a definite band of darkened bark appeared about all slits except the controls.

Young green York Imperial apples inoculated with Peach I through needle punctures developed after 28 days black areas 2 to 5 mm. in diameter surrounded by yellow halos extending 5 to 15 mm. farther out. The results with apple fruits were similar to those reported by the writer (11) for a species that he called *Phomopsis mali* and shown by Kidd and Beaumont (8) to belong in the same species group as the forms that they isolated from drupaceous and pomaceous fruits in Britain.

TAXONOMIC CONSIDERATIONS

Saccardo's description of *Phoma persicae* suggests that it is a *Phomopsis*, but, without examining type specimens, it cannot be stated definitely that it is, nor can one be certain whether or not Selby (12) was correct in considering the constriction-disease pathogen identical with *Phoma persicae*. The spore dimensions of *Phoma persicae* are essentially the same as those of the α spores of the constriction-disease pathogen.

In Europe, and especially in England, a species of *Diaporthe* with a *Phomopsis* stage has been given special attention because it causes the "die back" of stone fruits, a disease similar to or identical with the constriction disease (2, 3, 4). The "die back" disease also attacks branches of weakened or devitalized trees, causing a wilting of the leaves. Cayley (3) showed that this disease was caused by species of *Diaporthe* that fruited on the branches following a *Phomopsis* stage that produced both α and β spores. Inoculations with pure cultures were successful, especially on the peach. She iden-

tified the fungus as *Diaporthe perniciosa* March., an identification that has been accepted by European workers (2, 8, 9). Kidd and Beaumont (8) have shown that *Phomopsis mali*, described from apple by the writer (11), is identical with the *Phomopsis* stage of *D. perniciosa*, and it is the writer's opinion that *Phomopsis mali* is identical with the isolates from the constriction-disease cankers and the isolates from other fruits described elsewhere in this paper. Dunegan (5) also has produced evidence indicating that the ascogenous stage of *P. mali* is *D. perniciosa*. Since the writer has not found an ascogenous stage, either in peach branches or in artificial cultures, he cannot be certain that the pathogen is identical with *D. perniciosa*, but the available evidence strongly supports this view.

Although European investigators consider that the species of *Phomopsis* commonly occurring on drupaceous and pomaceous fruits is the pycnidial stage of *Diaporthe perniciosa* (2, 3, 8, 9), Wehmeyer (14) places it and *D. perniciosa* under *D. eres*. In a discussion of *D. eres* he states (p. 64), "A careful and intensive study of both stages of these related forms will undoubtedly reveal many minor varieties which are limited to certain hosts, but there exists at present such a maze of transitional forms that it is extremely difficult to determine any specific lines of separation on a morphological basis." Certainly it would be impossible to distinguish species of *Phomopsis* among the isolates used in the writer's experiments. Although there are some differences that can be noted, the differences between Peach I and Peach III are as great as those between any other possible pairs, despite the fact that Peach I and Peach III were isolated from similar lesions on branches taken from the same clump of trees. Marsh and Nattrass (9) noted considerable differences in cultural characteristics among their isolates, especially in the formation of fruiting bodies. Grove (6) is of the opinion that the "die back" fungus is a form of one or more previously known species.

The pathogen, then, may be classified as *Diaporthe perniciosa* March., if one considers the forms on fruit trees a distinct species, or as *D. eres* Nit., if he considers the forms on fruit trees as belonging to a larger species complex. Because of Wehmeyer's extensive work with these forms, the writer is inclined to accept his judgment and consider the pathogen as a form of *D. eres*.

SUMMARY

The constriction disease of peach described by Selby in 1898 was found in Delaware causing serious injury to weakened or previously injured trees. It is considered a disease of minor importance.

The pathogen that has been called *Phoma persicae* is shown to be a species of *Phomopsis*, as suggested by Anna E. Jenkins in a note on a herbarium sheet in 1915. It is similar to and possibly identical with forms isolated from branches of sweet cherry, apple, and pear. The disease is apparently identical with and caused by the same fungus as the disease known as "die back" in Europe.

The isolates from constriction disease of peach caused the disease when inoculations were made through burned areas, and were apparently somewhat more parasitic than *Phomopsis* isolates from other species of fruit trees.

A *Diaporthe* stage has not been found either in culture or on peach branches. However, reasons are given for considering the pathogen as identical with *Diaporthe perniciosa* and belonging to the species complex that Wehmeyer classifies as *Diaporthe eres*.

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CEPHALOSPORIUM LEAF SPOT OF TWO AROIDS

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(Accepted for publication May 8, 1940)

In the summer of 1938 the writer's attention was called to a severe leaf spotting of *Nephtytis afzelii* Schott (*N. liberica* N.E.Br.) and *Syngonium podophyllum* Schott var. *albolineatum* Engl. in a greenhouse on Staten Island, New York. Since the costae and lateral nerves in the latter plant are whitish, it is commonly designated by florists as "variegated *Nephtytis*." Both plants belong in the Araceae. In addition to being grown as house plants, they are sometimes featured in so-called "tropical displays" in store windows. Since they are cultured principally for their foliage, a few leaf spots are sufficient to reduce considerably their salability.

SYMPTOMS

The symptoms of the disease on both susceptibles are characterized by small, reddish-brown, circular to irregular, necrotic leaf spots surrounded by pale yellow borders (Fig. 1, A, B). These lesions vary in diameter from less than 1 mm. up to 5-6 mm. Sometimes the lesions coalesce, involving comparatively large areas of the leaves. As the spots enlarge, the centers become grayish in color and papery in texture. If infection is severe, the leaves turn yellow and die. Reddish brown, elongated lesions from 1 to 3 mm. in length occasionally are found on the petioles.

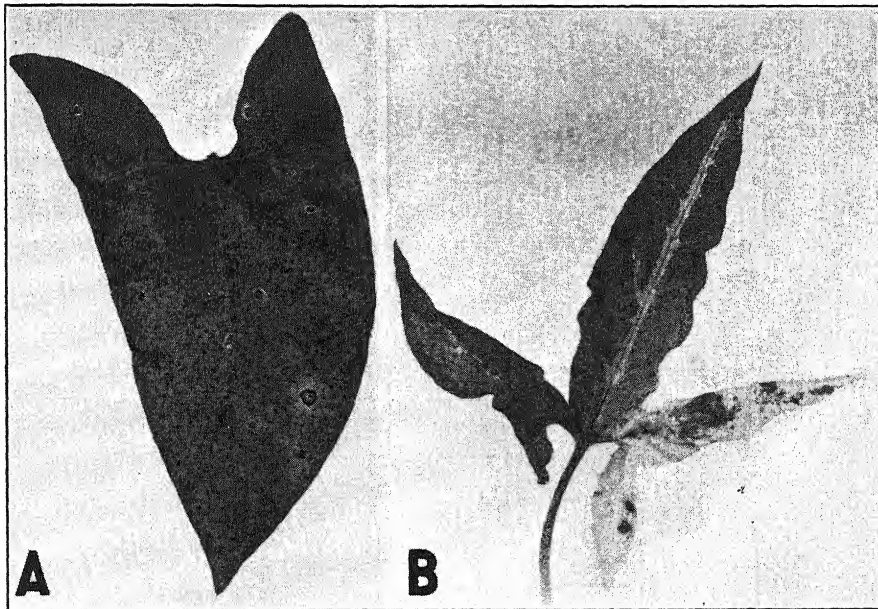


FIG. 1. Symptoms of *Cephalosporium* leaf spot. A. A naturally inoculated leaf of *Nephthytis afzelii*. B. An artificially inoculated leaf of *Syngonium podophyllum* var. *albolineatum*. Complete yellowing of one of the lateral lobes had resulted from a lesion on the vein.

PATHOGENICITY

A species of *Cephalosporium* has been repeatedly isolated from lesions on the leaves and petioles of both susceptibles and its pathogenicity established. The results of cross inoculations, with isolates from *Nephthytis afzelii* and *Syngonium podophyllum* var. *albolineatum*, show that the same fungus causes the disease on both susceptibles. Artificial inoculation was accomplished by placing blocks of agar bearing the pathogen on healthy leaves of the susceptibles, or by atomizing spore suspensions on the leaves. Plants so inoculated and kept under bell glasses, at approximately 100 per cent relative humidity and at from 20° to 27° C. for 18 hours, show symptoms of infection within 24 to 36 hours. The first symptoms in such instances are irregular hydrotic areas from 1 to 3 mm. in diameter. After removal of the bell

glasses, the hydrotic condition disappears leaving necrotic spots as previously described.

The results of experiments, in which both upper and lower leaf surfaces were inoculated with spore suspensions, indicate that both surfaces are readily penetrated as determined from the number of resulting lesions. Twelve hours after inoculation one of these leaves was flooded with cotton blue stain. Examination under the microscope showed that the germ tubes, which arise either laterally or terminally from the spores, seem to penetrate directly through the epidermis by means of peg-like tubes. There was no evidence of stomatal invasion.

There is a slight tendency for infection to be systemic in both *Nephtytis* and *Syngonium*, as evidenced by the fact that if a large lesion develops at the point of attachment of the leaf blade to the petiole, the pathogen can be recovered subsequently from the upper one-third of the petiole. Spread down the petiole into the stem has not been observed. If the petiole is wounded and then inoculated, yellowing and death of the leaf follows within 3 to 5 days. One-sided yellowing of the divided leaf of *Syngonium* may occur if a lesion develops on the main vein of a lateral leaf lobe (Fig. 1, B).

The results of experiments in which young leaves (less than 1 month old) and older leaves (more than 2 months old) of *Nephtytis afzelii* were inoculated with spore suspensions show that the young leaves of this species are somewhat more susceptible to infection than are the more mature ones.

THE PATHOGEN

The pathogen, on 2 per cent potato-dextrose agar, forms a whitish growth that soon becomes cinnamon-buff,¹ and varies in form from fluffy to appressed and watery. A network of radiating strands of hyphae is sometimes formed. The branched, septate mycelium produces lateral, simple to compound, continuous to septate conidiophores that taper near the apex (Fig. 2, A, B). Conidiophores forming on detached leaves in moist chambers are mostly unbranched. In culture, short, secondary conidiophores may be produced, which occasionally branch in whorls of 3 or 4 (Fig. 2, B). The tapering is more pronounced in the compound than in the unbranched conidiophores. The conidia are abstricted singly at the tip of the conidiophore, each newly-formed spore being pushed aside by the next one produced. Those forming on upright, aerial conidiophores are for the most part continuous to 1-septate, ovoid to short cylindric with rounded ends, and are aggregated in spherical, glistening, and grayish-white slimy heads (Fig. 2, C) that break apart readily when mounted in water. Conidia forming on decumbent conidiophores, and in contact with the surface of the agar, are mostly 1- to 4-septate and cylindrical with rounded ends. Both terminal and intercalary chlamydospores are formed in culture (Fig. 2, D).

¹Ridgway, R. Color standards and color nomenclature. 43 pp. (Washington) 1912.

The minimum temperature for growth on potato-dextrose agar is 9° C., the optimum between 24° and 27° C., and the maximum, 30°.

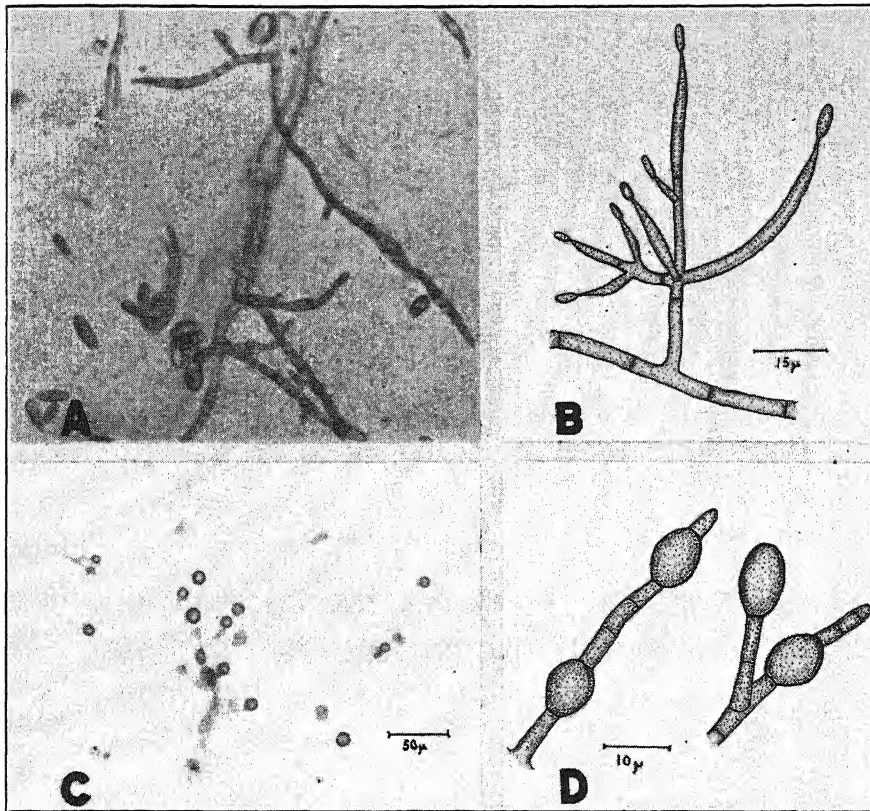


FIG. 2. *Cephalosporium cinnamomeum*. A. Conidiophores with lateral branches. B. Compound conidiophore showing branching, septation, and conidia. C. Mucous spore heads borne on aerial conidiophores on potato-dextrose agar. D. Terminal and intercalary chlamydospores formed in culture.

TAXONOMY

The species of *Cephalosporium* causing the leaf-spot disease on *Nephtytis afzelii* and on *Syngonium podophyllum* var. *albolineatum* is apparently unlike any other previously described. However, the descriptions of *Cephalosporium* spp. in the standard references, e.g., Saccardo's *Sylloge Fungorum*, are so brief and incomplete that adequate comparisons are difficult. The *Cephalosporium* described in this paper resembles most *C. pammellii* Buchanan,² but differs from it in that (1) neither allantoid nor sickle-shape spores are produced, (2) the conidiophores taper near the apex while those of *C. pammellii* are straight, and (3) chromogenesis (cinnamon-buff) occurs in culture as contrasted with absence of color in *C. pammellii*.

² Buchanan, R. E. Morphology of the genus *Cephalosporium*, with description of a new species and a new variety. Mycol. 3: 170-174. 1911.

In view of this and the fact that no species of *Cephalosporium* or morphologically-similar fungi (*Acrostalagmus*, *Hyalopus*) have been previously reported on these 2 suspects or on related plants, the writer feels it is best to regard this as a new species and proposes for it the name *Cephalosporium cinnamomeum*, sp. nov.

Technical Description of the Pathogen

Cephalosporium cinnamomeum, sp. nov. Hyphae sterile, creeping, fluffy, to appressed and watery, white soon becoming cinnamon-buff, septate, branching, 2-4 μ thick; chlamydospores formed both terminally and intercalarily, 7-10 \times 12-14 μ ; conidiophores arising laterally from the mycelium, erect or decumbent, simple and continuous to compound and septate, tapering near the apex, 1.6-2.8 \times 3.5-45 μ producing conidia in mucous heads which are 8-18 μ in diameter; conidia from erect, aerial conidiophores, ovoid to short-cylindric, none to 1 septum, 1.5-5.8 \times 4.2-15 μ , those from decumbent conidiophores mostly cylindric, 1-4-septate, up to 22.6 μ long.

Type material, including infected leaves of *Nephtytis afzelii* and *Synгонium podophyllum* var. *albolineatum* and dried agar cultures of the pathogen, has been deposited in the Herbarium of the Department of Plant Pathology of Cornell University (No. 27015).

CONTROL

Preliminary experiments indicate that fairly satisfactory control of the disease on *Nephtytis afzelii* may be obtained by spraying with Cuprenox³ (copper oxychloride) diluted at the rate of 1 gallon in 100 gallons of water. Other materials, tested and found to be undesirable because of unsightly residues on the leaves, are Bordeaux mixture (2-1-50), Kolodust, and colloidal sulphur. Further experiments with these and other fungicides are now under way.

Maintaining greenhouse temperature and humidity as low as possible, and avoiding splashing water on the leaves of the plants are recommended.

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CUCURBIT DISEASES AND ROT OF TOMATO FRUIT CAUSED BY PHYTOPHTHORA CAPSICI¹

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(Accepted for publication May 8, 1940)

INTRODUCTION

Serious crop losses in cucurbits and tomatoes have occurred during the past 4 years in the Arkansas River Valley of Colorado. Preliminary studies indicated that much of this damage was due to diseases caused by *Phytophthora capsici* Leonian.

³ This material was obtained from the Hooker Electrochemical Company, Niagara Falls, N. Y. It is not available commercially in this country at the present time.

¹ Published with the approval of the Director of the Colorado Agricultural Experiment Station.

² The writers wish to acknowledge the assistance of Dr. C. M. Tucker of the Department of Botany of the University of Missouri, who verified the determinations of the identity of all isolates reported herein.

For nearly a decade, pepper blight, caused by *Phytophthora capsici*, has been a limiting factor in the pepper production of the above named region. It was first observed there by Bodine³ in 1931, when the causal agent was isolated and its pathogenicity to peppers established. In this same publication it was mentioned that infection of eggplant (*Solanum melongena* L. var. *esculentum* Nees) by *P. capsici* was found in this region. In 1937 Kreutzer⁴ reported a rot of cucumber fruit (*Cucumis sativus* L.), present in the Arkansas River Valley, was induced by a species of *Phytophthora*. This isolate was shown to be capable of causing typical pepper blight. Two years later, Wiant⁵ reported his obtaining cultures of *Phytophthora* from decaying Honeydew melon (*Cucumis melo* L. var. *inodorus* Naud.) and cantaloupe fruits (*Cucumis melo* L. var. *reticulatus* Naud.), which had been shipped to the New York market from that part of Colorado. The majority of these cultures were identified by C. M. Tucker, University of Missouri, as isolates of *P. capsici*.

The only pertinent report having to do with material not of Colorado origin is that of Tompkins and Tucker⁶ in 1937. These investigators found that a rot of Honeydew melon fruits occurring in the San Joaquin Valley, California, was induced by *P. capsici*.

THE DISEASES

Decay of Cucumber Fruit

In the summer of 1936 a *Phytophthora* was obtained from decaying cucumber fruits previously collected in considerable quantity in a field in the Rocky Ford region of the Arkansas River Valley.⁴ The disease was epiphytotic in the area. Affected fruits were soft and discolored, revealing water-soaked and olive to yellow, sunken areas. When the fruits were broken open, the rotting pulp gave off a marked odor of fermentation. As a rule, signs of the disease were absent, except in cases where the fruits were lying in wet spots in the fields. The vines apparently were unaffected by the organism.

When clean, green, or ripe cucumber fruits were placed in moist chambers and exposed to infection by placing a small piece of fungus inoculum upon the uninjured cuticle, a rot ensued (Fig. 1, B). The affected fruits were soon reduced to slimy viscous masses held in place only by the epidermis and cuticle. Because of its morphological similarity in culture to known cultures of *Phytophthora capsici* on hand, the fungus was introduced into soil in which 9-week-old pepper transplants of the varieties Ruby King and

³ Bodine, E. W. Blight of peppers. Colorado Experiment Station Press Bull. 85. 1935.

⁴ Kreutzer, W. A. A *Phytophthora* rot of cucumber fruit. (Abstract) *Phytopath.* 27: 955. 1937.

⁵ Wiant, James S. Species of *Phytophthora* responsible for market decay of western Honey Dew melons and cantaloupes. *Pl. Dis. Rptr.* 23(19): 322. 1939.

⁶ Tompkins, C. M., and C. M. Tucker. *Phytophthora* rot of Honeydew melon. *Jour. Agr. Res. [U. S.]* 54: 933-944. 1937.

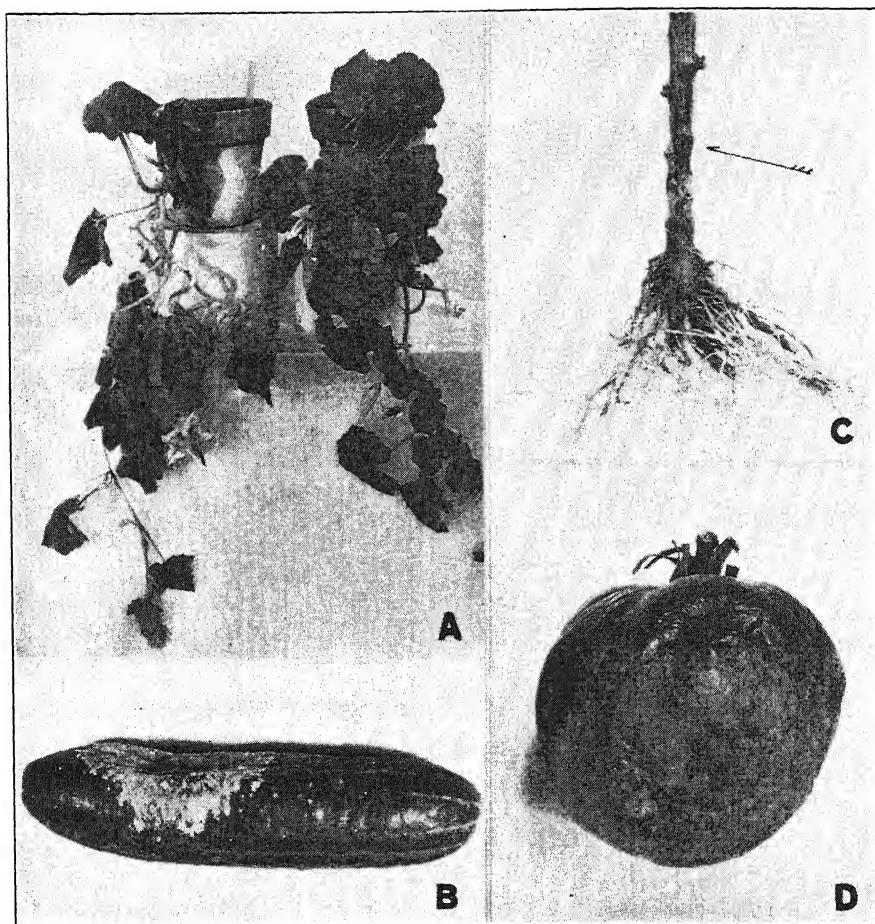


FIG. 1. Diseases caused by *Phytophthora capsici*. A. Wilt of squash. The plant on the left shows typical symptoms. Control plant on the right for comparison. B. Rot of cucumber fruit (artificial inoculation). C. The stem of a diseased pepper plant showing the characteristic basal stem lesion induced by the pathogen (specimen taken from the field). D. Rot of tomato fruit (field specimen).

California Wonder were growing. Care was taken not to injure the plants in any case. Wilt was induced in from 4 to 8 days, followed by the appearance of pronounced stem lesions (Fig. 1, C) and the death of the plants within 15 days. It was observed that inoculum placed just adjacent to the stem of the pepper plant was more rapid in its effect than that introduced into the soil several inches from the stem.

Rot of Tomato Fruit

A serious rot of tomato fruit (*Lycopersicum esculentum* Mill.) occurring in the Arkansas River Valley in the vicinities of Rocky Ford and Manzanola was called to the writers' attention in August, 1938. Fruits, decayed on the vines, became soft and discolored (Fig. 1, D).

Tissue plantings on nutrient agar from decaying fruits uniformly yielded cultures of a *Phytophthora*. The organism was isolated both from rotting fruits picked from vines in the field and from fruits obtained from stores. Both cultures proved pathogenic to uninjured ripe tomato fruits. Again, because of cultural similarity to cultures of *P. capsici*, the isolates were introduced into the soil in which 8-week-old pepper plants of the variety California Wonder were growing. The majority of the plants so treated wilted in 6 days and were dead in 14 days.

The epiphytotic of tomato fruit rot in 1938 was preceded by an unusual amount of precipitation, and the late summer and early fall rains so aggravated the malady that, by fall, the major canning companies in this area were considerably alarmed by the enormity of the tomato-crop losses. During the summer of 1939 very little fruit-rot was observed, probably because of the comparatively dry growing season, which precluded any extensive spread of the disease.

Wilt of Squash and Watermelon Vines

Recent studies have shown that a *Phytophthora* was responsible for a wilt of squash (*Cucurbita maxima* Duc.) in the Arkansas River Valley in the summer of 1938. This isolate was obtained from lesions on the basal portions of stems of wilted plants. When introduced into soil in which uninjured 7-week-old Hubbard squash plants were growing, the fungus induced a severe wilt in 6 days (Fig. 1, A).

Several weeks later, isolations from the basal stem lesions of wilted watermelon plants (*Citrullus vulgaris* Schrad.) in the vicinity of Greeley, Colorado, yielded cultures of a fungus that were similar to those previously obtained from the diseased squash plants. This isolate proved capable of causing a pronounced wilt of uninjured 6-week-old Kleckley Sweet watermelon plants, in a period of 7 days. It was found that the watermelon isolate was capable of inducing a wilt of squash, and, in like fashion, the organism obtained from squash was found capable of producing watermelon wilt. No marked differences in virulence were observed.

Since neither of these isolates showed any morphological differences in culture from those cultures previously described in this report, an investigation was conducted to determine whether they were pathogenic to mature uninjured pepper plants. Such plants growing in soil infested with either isolate wilted in 6 days and were dead within 14 days. All plants subjected to infection showed the stem lesions characteristic of pepper blight.

Identity of the Causal Agent

Because all isolates reported herein showed morphological similarities in culture to isolates obtained from blighted pepper plants, and since all were uniformly pathogenic to mature, uninjured pepper plants, causing typical pepper blight symptoms, they were tentatively identified as *Phytophthora*

capsici Leonian. Cultures of these isolates were subsequently submitted to C. M. Tucker of the University of Missouri, who verified the determinations.

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PHYTOPATHOLOGICAL NOTES

Field Observations on the Dying of Pines Infected with the Blue-stain Fungus, Ceratostomella pini Münch.—This note was prompted by reading the preceding article by W. C. Bramble and E. C. Holst describing results following their inoculation of shortleaf pines with certain fungi associated with the southern pine beetle.

The writers were closely associated with these men as their studies progressed, and during 1934 there was an excellent opportunity to obtain data on trees dying in nature following blue-stain inoculations resulting from unsuccessful attacks by bark beetles. This is now known to occur commonly from 1 to 3 years after an aggressive outbreak of bark beetles has subsided. The field observations made near Fairfax, Virginia, and reported herein very effectively support the experimental work of Bramble and Holst.

The infestation of the southern pine beetle from which these notes were obtained began during the spring of 1931 following the severe drought of 1930 and the drying up of local streams. The following summer the infestation spread rapidly, killing much of the timber in this area. The situation was aggravated by the continued drought and mild winters during this period. The ample rains of the spring of 1933 partly checked the epidemic, but it was not until the winter of 1933-34 that prolonged subzero temperature brought about complete natural control.

During the summer and fall of 1933 many shortleaf pines were attacked by bark beetles, but, because of the increased resin flow, these attacks were unsuccessful and the beetles were "pitched out" and killed before they could construct their egg tunnels. Relatively few beetles reached the xylem in comparison with the number in trees successfully attacked during the height of an outbreak. Normally, attacked trees die rapidly as a result of blue-stain penetration and the rapid drying accompanying the complete destruction of the phloem; and the foliage shows indications of fading in about 3 weeks' time. These unsuccessfully attacked trees did not fade, however, until the following spring, as the volume of blue stain developing from the smaller number of beetle contacts on the surface of the xylem was unable quickly to cut off conduction.

Several of these trees were examined on May 27, 1934, when the foliage was fading. The results, presented in table 1, show that blue stain coincided with the attacked portions of the stems of the trees, although almost no brood developed in any of them. These trees represent a condition that existed in other parts of the area and has subsequently been found to be typical of

TABLE 1.—Correlation of blue-stain development with attack on shortleaf pine by *Dendroctonus frontalis* Zimm.

Tree			Beetle attack (fall of 1933)				Blue-stain development, height on stem and extent
No.	Diameter, breast height	Height	Color of foliage after attack	Height on stem	Brood development		
					Successful	Unsuccessful	
1.	<i>Inches</i> 14	<i>Feet</i> 70	Sorrel	<i>Feet</i> 10 to 40	South side; only a few larvae and pupae alive	North side, no brood, adults dead in 1-inch tunnels	<i>Feet</i> 10 to 40; stain all around and penetrating into heartwood
2.	14	67	Pale green	5 to 43, mostly south side	Only a few pupae survived	Adults and small larvae dead	5 to 43; stain mostly on one side, except at 30 ft., where all around; into heartwood where present
3.	11	65	Sorrel	3 to 43	None	Adults dead in 3-inch tun- nels, tiny larvae also dead	3 to 43; nearly all around and into heartwood
4.	13	65	Sorrel	3 to 45	None	Adults dead in extended tun- nels one-fourth around. Many tunnels only begun	3 to 45; nearly all around and into heartwood
5.	10	60	Sorrel	3 to 45, fairly heavy attack at mid- stem	None	3 ft.; pitched out	3; none
						10-15 ft.; dead adults and attack only one-fourth around	10-15; to heartwood one- fourth around
						20 ft.; dead adults three fourths around	20; to heartwood three- fourths around
						30 and 40 ft.; as 10 ft. and 3 ft. respectively	30 and 40; as at 10 ft. and 3 ft., respectively

TABLE 1.—Continued

No.	Tree		Color of foliage after attack	Height on stem	Beetle attack (fall of 1933)		Blue-stain development, height on stem and extent
	Diameter, breast height	Height			Successful	Brood development	
	<i>Inches</i>	<i>Feet</i>				Unsuccessful	
6.	9	68	Pale green	<i>Feet</i> 5 to 25, light	None	Dead adults, one-quarter-one-half around, tunnels to 1 inch long	<i>Feet</i> Stain present where attacked; at 10; all around
7.	9	65	Pale green	3 to 20, light	None	Dead adults; in narrow strip at 3 feet to one-half around at 20 feet	Stain present in region of attack
8.	8	45	Greenish yellow	3 to 20, heavy	None	Dead adults all around in partially excavated tunnels	Stain to heartwood in region of attack
9. ^a	15	70	Pale green	10 to 25, heavy	None	Dead adults all around in partially excavated tunnels	Stain to heartwood in region of attack

^a Virginia scrub pine.

the end of each outbreak.—F. C. CRAIGHEAD and R. A. ST. GEORGE, Bureau of Entomology and Plant Quarantine, U. S. Dept. Agr., Washington, D. C.

Occurrence of Scolecospore-producing Strains of Diplodia zeae in the United States.—In an earlier paper¹ the writer reported the occurrence of scolecospores in a single strain of *Diplodia zeae* isolated in 1934 from a rotted corn kernel from Ohio and stated that "No information is at hand concerning the distribution of such strains in the Corn Belt."

Since that time surveys based on plated kernels from some of the samples of *Diplodia*-rotted corn gathered by Hoppe² in his annual surveys of the fungi present in damaged corn indicate that scolecospore-producing strains of *Diplodia zeae* are widely distributed and common in the corn growing regions of the United States. With a few exceptions the samples used were limited to damaged grain from carload lots of corn arriving at a number of terminal markets in the midwestern and eastern sections of the country in January and February of 1939 and 1940.

Scolecospores appeared on the surface of plated kernels from 179 samples of corn shipped from 14 States. This count recorded only those kernels on whose surface spore masses appeared that were visible to the naked eye or could be recognized with the aid of a hand lens. (Table 1.)

TABLE 1.—Location and number of shipping points from which samples of damaged corn containing scolecospore-producing strains of *Diplodia zeae* were obtained in January and February 1939 and 1940

State	Year	Number of		
		Counties	Towns	Samples
Alabama	1939	1	1	2
Delaware	1940	2	3	4
Illinois	1939	14	28	31
Do	1940	21	36	38
Indiana	1939	7	13	17
Do	1940	8	11	18
Iowa	1939	12	13	13
Do	1940	12	17	18
Kansas	1939	1	1	1
Kentucky	1939	1	2	2
Maryland	1940	6	8	16
Minnesota	1939	2	2	2
Do	1940	5	6	6
Missouri	1939	1	1	2
Do	1940	1	1	1
Ohio	1939	2	2	2
Do	1940	3	3	3
South Carolina	1940	1	1	1
Tennessee	1939	1	1	1
Wisconsin	1940	1	1	1
Total		92 ^a	145 ^a	179

^a Duplicates not included.

¹ Johann, Helen. Scolecospores in *Diplodia zeae*. Phytopath. 29: 67-71. 1939.

² Hoppe, P. E. Relative prevalence and geographic distribution of various ear rot fungi in the 1938 corn crop. Plant Dis. Rptr. 23: 142-148. 1939.

The method by which they were obtained was as follows: *Diplodia*-infected kernels, after surface treatment with BK, were placed on a fairly thick layer of potato-dextrose agar, 4 to 7 kernels per plate, incubated under ordinary laboratory conditions until the surface of the agar had been covered with hyphae, after which the Petri dishes were stored in a moist chamber for 3 to 10 weeks. The ivory to tan-color spore masses emerged in the form of droplets or spore horns, depending on the amount of moisture present. Usually, they were seen first near the distal end of the kernels

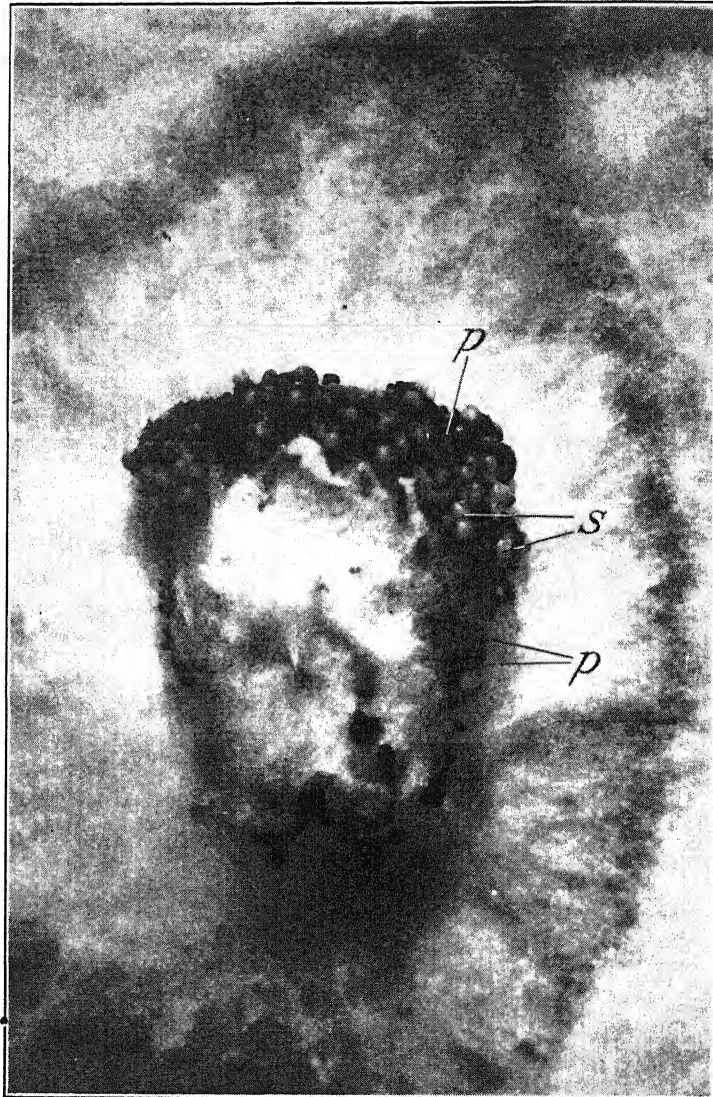


FIG. 1. *Diplodia*-infected kernel from which bicellular brown pycnospores and scolecospores have emerged. Kernel plated on potato-dextrose agar. s. Masses of ivory-color scolecospores. p. Pyrenidia containing bicellular brown pycnospores.

among the pycnidia discharging brown spores, and often were in great abundance on the sides and crown of the kernels (Fig. 1). The fruiting bodies containing each of the 2 types of spores were much alike in appearance. They were superficial or more or less imbedded in the kernel, massed, or solitary.

A small number of the scolecospores have been germinated in hanging drops. When transferred to agar plates the resulting colonies resembled in appearance those produced from the bicellular brown pycnospores, and the former, as well as the latter, have produced the brown pycnospores characteristic of *Diplodia zeae*.—HELEN JOHANN, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, University of Wisconsin, Madison, Wis.

Wilt of Salsify, Caused by Verticillium sp.—In late autumn of 1939, H. H. Whetzel collected in his garden at Ithaca, New York, and brought to the laboratory, several roots of salsify (*Tragopogon porrifolius* L.) that showed a brownish discoloration of the vascular tissue. On microscopic examination the vessels were found to contain an abundance of fine mycelium. A species of *Verticillium* characterized by the production of both torulose hyphae and "sclerotia" was readily isolated from the diseased specimens. This is interesting because the species *Verticillium dahliae* Kleb. and *V. alboatrum* R. and B. have often been distinguished on the basis of the presence or absence of "sclerotia."

The first report of a *Verticillium* in salsify was made by Jagger and Stewart¹ who collected material in the vicinity of Rochester, New York. The authors described the symptoms of the disease as a gradual wilting and dying of the outer leaves and a greyish-brown discoloration in the xylem of the roots and rootlets. The fungus isolated from the diseased material proved to be *Verticillium* sp. Apparently, no inoculations were made to prove the pathogenicity of the fungus.

The brief note by these workers is the only report we have on *Verticillium*-wilt of salsify, and all further references made to the disease go back to their work.

The pathogenicity of the *Verticillium* from the naturally-infected salsify roots was studied. Inoculum was prepared following largely the method as outlined by Wellman.² The *Verticillium* isolated from salsify was allowed to grow on the Tochinai medium for 10 days, during which period it made excellent growth. The mycelium was then separated from the liquid and beaten up in sterile distilled water. The roots of the plants to be inoculated were dipped into this suspension. The salsify plants to be used in the tests had been grown from sterilized seed in sterile soil. When the seedlings had reached the age of 3 weeks they were removed from

¹ Jagger, J. C., and V. B. Stewart. Some *Verticillium* diseases. *Phytopath.* 8: 15-19. 1918.

² Wellman, F. L. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. *Phytopath.* 29: 945-956. 1939.

the flats, inoculated and transferred to pots, 3 plants usually being planted in each pot. In order to avoid injuries to the roots the plants were actually "washed" out of the flats, thus freeing them from the adhering soil. Inoculation was made by dipping the roots into the above described suspension containing bits of mycelium and spores of the fungus. Part of the seedlings, not dipped, served as a control. All pots were moved to a greenhouse maintained at approximately 72°-78° F.; on sunny days the mercury sometimes rose to 85° F.

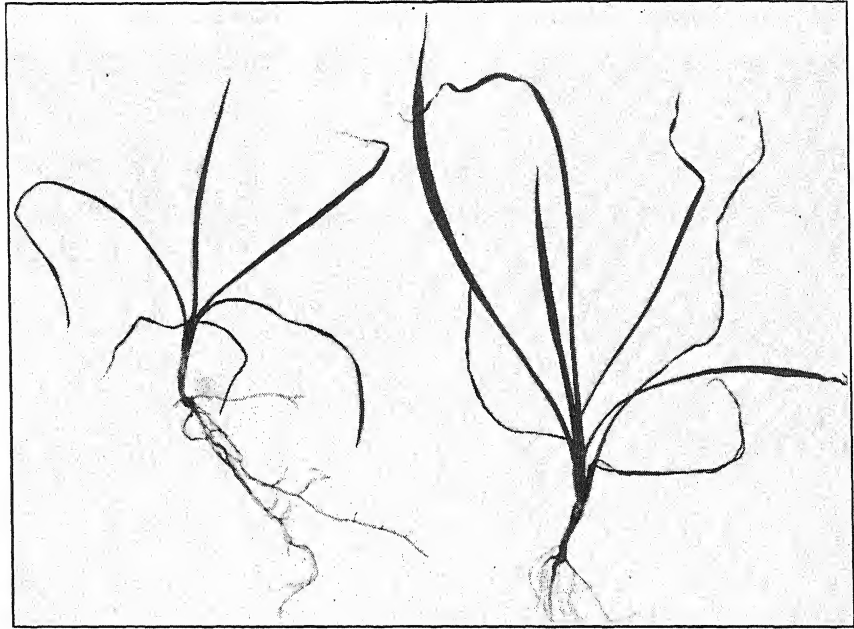


FIG. 1. Seedlings of *Tragopogon porrifolius* L. inoculated with a culture of *Verticillium* sp. isolated from naturally infected salsify.

During the first month following inoculation 12 of the 25 inoculated plants showed wilting beginning with the outer leaves (Fig. 1). The tips shriveled first but soon the entire leaf became involved and then faded from green to greyish, yellowish, and brown. On the roots, no external lesions were noticeable, but the vascular tissue became discolored, varying in shades of reddish-brown and greyish-brown. Microscopic examination revealed the presence of a fungus growing in the xylem. A *Verticillium* identical with that used for inoculation was reisolated from the roots. The other 13 plants appeared still healthy 3½ months following inoculation, and no fungus growth was found in their roots.

The fact that only 12 out of 25 plants had become infected seems puzzling. Lack of inoculum cannot be the reason, since the same treatment was given to all of the plants to be inoculated. It was observed that the diseased plants were well distributed in all of the pots, which indicates that in each

pot inoculum was present. It is possible that this failure to procure infection of all the plants may be because of inability of the pathogen to enter uninjured tissue. It appears possible that some of the rootlets may have been broken, no matter how carefully the plants were handled, and that these injured plants alone could be invaded by the pathogen.—KARLA LONGRÉE, Dept. of Plant Pathology, Cornell University, Ithaca, N. Y.

*Control of Cedar Rust with Sodium Dinitrocresylate.*¹—Sodium dinitrocresylate (Elgetol), applied as a single spray on May 8 to a group of red cedar trees and on May 16 to another group when rust galls were showing signs of activity, inhibited telial column extension and teliospore germination from rust galls of *Gymnosporangium globosum*, and *G. juniperi-virginianae*, on red cedar, *Juniperus virginiana* and its varieties, and *Gymnosporangium clavipes*, and *G. clavariaeforme* on the dwarf juniper, *J. communis*.

A 1 per cent solution of Elgetol regular was most effective. Weaker strengths did not penetrate so deeply and allowed some later extension of telia. An exceptionally thorough spraying was necessary for positive results.

No injury to foliage was apparent after 58 and 50 days, respectively, on any of the red cedar trees, but a trace of injury was discernible on some of the badly infected dwarf junipers. Twenty-six red cedar trees and 10 clumps of dwarf junipers were used in the tests. Several hundred nonsprayed trees served as controls.

Gymnosporangium globosum galls predominated. It was estimated that some of the red cedar trees had as many as 8,000 to 12,000 galls per tree. In some instances only the lower half of the tree was sprayed. Complete cessation of telial extension was evident on the sprayed portion, while on the nonsprayed upper portion of the tree, telia developed normally and, when wet, produced the mass of yellow jelly-like material typical of this fungus.

This appears to be the most effective fungicidal compound yet tried on the telial stage of the *Gymnosporangium* rust fungi. The prevention of the germination of teliospores and production of sporidia forestalls infection of the pomaceous hosts and thus gives a new method of attack for the control of the cedar rust fungi. The authors feel that the results of these tests warrant the trial of this method of control of the *Gymnosporangium* rusts on a much larger scale next season.—FORREST C. STRONG, and DONALD CATION, Michigan State College, East Lansing, Mich.

¹ Michigan Agricultural Experiment Station journal article No. 447 (n.s.) June 3, 1940.

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONSTITUTION

ARTICLE I

This Society shall be known as The American Phytopathological Society.

ARTICLE II

Sec. 1. The Society shall consist of members, and may include life members and patrons.

Sec. 2. The charter membership of this Society shall consist of the one hundred and thirty persons who accepted the invitation of the Organization Committee of October 25, 1909, to form the Society.

ARTICLE III

QUALIFICATIONS FOR MEMBERSHIP AND DUES

Sec. 1. All persons interested in the study of phytopathology, including the practical control of plant diseases, shall be eligible to membership.

ARTICLE IV

ELECTION OF MEMBERS

Members may be elected at any regular meeting of the Society or by the Council during the interim. Applications for membership must be endorsed by at least one member of the Society.

ARTICLE V

OFFICERS

The officers of the Society shall consist of a President, Vice-President, Secretary, and Treasurer. Their duties shall be those usually performed by such officers. The President and Vice-President will serve for one year or until their successors are elected, and the Council shall fill any vacancies occurring between annual meetings. The Secretary and Treasurer shall be appointed by the Council for a term of 3 years and the Council shall fill any vacancy occurring between annual meetings.

The Council shall consist of the President, Vice-President, Secretary, Treasurer, the retiring President, and the Chairman of the Board of Editors of the Journal of the Society, with two members elected one each year, who shall serve for a term of two years, and one member elected annually by each Division. The term of service of a Council member from a Division shall commence immediately following his election and shall continue until his successor is elected.

All action of the Council or officers must be authorized or approved by the Society.

ARTICLE VI

ELECTION OF OFFICERS

The Secretary shall send nomination ballots for officers to each member of the Society in time to allow all nominations to be returned not less than two months before the date of the annual meeting. The Council shall make nominations for any office when such nominations are wanting. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot, which shall be sent to each member one month before the annual meeting, provided, however, that, if the same person be found to have received sufficient ballots to qualify for nomination to more than one office, his name shall be placed on the final ballot as a candidate only for the highest of these offices as indicated in their order in Article V. Votes shall be mailed to the Secretary and canvassed by the Council. A plurality vote shall elect.

ARTICLE VII

EDITORS, COMMITTEES, AND APPOINTMENTS

The Editors of the official organ of the Society shall be selected by the Council subject to the approval of the Society.

Temporary or standing committees may be appointed at the discretion of the Society.

Unless otherwise directed, the President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies on standing committees that may occur during his term of office.

ARTICLE VIII

MEETING

An annual meeting shall be held at such time and place each year as the Council may select, and additional meetings, including special or local meetings, for the presentation of papers, may be arranged by the Council at its discretion.

Unless otherwise ordered, the Secretary and the President, or, in case of his inability to attend, the Vice-President, are authorized to attend the annual meetings of the Society at the Society's expense.

ARTICLE IX

DIVISIONS

Branch organizations or units within the Society, known as Divisions, may be established on a geographical basis, provided formal application setting forth the reasons for the establishment of the Division is made to, and approved by, the parent Society.

ARTICLE X

AMENDMENTS

This Constitution may be amended at any annual meeting by a three-fourths majority of all the members voting, notice of the proposed amendment having been sent to all the members at least one month previous to the meeting.

STANDING RULES

The rules under which The American Phytopathological Society operates are as follows:

PHYTOPATHOLOGY

1. The official publication of the Society shall be PHYTOPATHOLOGY.

a. *Officers.* The officers of PHYTOPATHOLOGY shall be an Editor in Chief, term three years; three Editors, terms three years; twelve Associate Editors, terms three years, four selected each year; Business Manager, term three years; and Advertising Manager, term one year.

b. *Selection of Officers.* The Editor in Chief and the Business Manager shall be selected by the Council and approved by the Society. The Editors and the twelve Associate Editors shall be selected by the Council in consultation with the Editor in Chief and approved by the Society. The Advertising Manager shall be selected by the Council in consultation with the Business Manager and approved by the Society.

c. *Subscriptions, Back Numbers.* Subscriptions to PHYTOPATHOLOGY for institutions and non-members shall be \$6 per year in the United States and dependencies; Canada, \$6.25; other countries, \$6.50. The price of current single numbers shall be 60 cents. The price of back volumes and numbers shall be determined by the Business Manager with the approval of the Council. Separate copies will not be sold except in cases where the volumes are already broken. Requests to supply lost copies of the journal without charge must be made within sixty days from date of issue.

DUES

2. The annual dues for each regular member, including subscription to PHYTOPATHOLOGY, shall be \$5 per year, payable on December 20. The Business Manager of PHYTOPATHOLOGY shall discontinue sending the journal to any members whose dues have not been received by December 20.

PAPERS, ABSTRACTS

3. Members who plan to present papers at the annual meeting must submit to the Secretary three copies of each abstract. These abstracts must be clear and concise, contain no tabular data, and not exceed 200 words in length. They should include only statements of fact, unpublished information, and directly derived conclusions or hypotheses. Reports of progress, or of disease occurrences, or of routine tests of ordinary control measures, are not desired, unless new and significant developments are clearly indicated.
 - a. *Date Due.* Abstracts must be received by the Secretary on or before November 1st. Members are requested not to submit abstracts unless they expect to attend the meeting.
 - b. *Number of.* Each member is limited to two papers on which he may appear as sole, senior or junior author.
 - c. *Editing and Reviewing of.* Abstracts are to be reviewed by a committee appointed annually by the Editor in Chief of PHYTOPATHOLOGY and this committee is directed to return to authors for revision such abstracts as fail to meet the above requirements.
 - d. *Time Limit on Papers.* Members are requested to limit presentation time to 5 to 10 minutes. The maximum time allowed for other than invitation papers will be 15 minutes. Complicated tables or graphs should not be shown.
 - e. *Publication of Abstracts.* Abstracts of papers presented at the annual meeting shall be printed in PHYTOPATHOLOGY at the expense of the Society.

PROGRAMS

4. The program for the annual meeting shall be in charge of a program committee consisting of the President, Vice-President, and Secretary. To relieve congestion the program committee is authorized to schedule simultaneous sessions when necessary.

DIVISIONS

5. The following provisions shall govern the organization and regulation of Divisions of the Society.
 - a. *Name of.* Divisions shall use the name of the parent Society with appropriate geographical term; for example, The American Phytopathological Society, Pacific Division.
 - b. *Membership.* Divisions shall elect to full membership only members of The American Phytopathological Society, but each Division may elect associate members under such rules as it may adopt.
 - c. *Publication.* The proceedings of Divisions shall be printed in PHYTOPATHOLOGY. The preliminary abstracts of the Division meetings may, at the discretion of these Divisions, be printed in PHYTOPATHOLOGY under the same rules that govern publication of abstracts of the general Society. This rule, however, shall not be interpreted as limiting the present right of the Editorial Board of PHYTOPATHOLOGY to define the character and amount of any manuscript for publication.
 - d. *Meetings of.* Whenever The American Phytopathological Society meets within the territory of a Division, the Division shall merge its program with that of the parent society. The scientific sessions of such a meeting shall be presided over

alternately by the President of The American Phytopathological Society and the President of the Division. Business sessions may be independent.

- e. *Constitution of.* The constitution or articles of organization of all Divisions shall contain a provision or provisions ratifying the above rules. The constitution of all Divisions shall contain nothing in conflict with the constitution of The American Phytopathological Society. With the exceptions defined by the above rules, the Divisions shall enjoy complete autonomy.

AUDITING COMMITTEE

7. At each annual meeting the President shall appoint an auditing committee to audit the accounts of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY.

BIOLOGICAL ABSTRACTS

8. The Society shall provide for a standing committee of five with terms of five years, one member being chosen each year, to cooperate with the Board of Editors of *Biological Abstracts* and the Union of American Biological Societies. The Editor in Chief and the Secretary shall be members *ex-officio*.

NATIONAL RESEARCH COUNCIL

9. Representation on the Division of Biology and Agriculture of the National Research Council is provided for in the following way. The various societies represented in the Division are classified into groups. This Society, the Society of American Bacteriologists, and the Mycological Society of America constitute Group V. This group, as is the case with the others, likewise is entitled to one representative. Each Society shall designate an elector and an alternate for three-year terms. The two electors are to choose the representative on the Division, for Group V, either one of themselves, or a member of one of the societies, the societies being taken in rotation. The term of the representative shall be three years. The elector from a Society not having the representative on the Division in a given period, or some other member whom the Society may select, shall serve as an advisory representative without vote, and without expenses paid by the National Research Council, and may attend divisional meetings to present directly any business of his society.

The elector and alternate of The American Phytopathological Society, terms three years, shall be selected by the Council of the Society and approved by the Society.

OTHER REPRESENTATIVES

10. The following shall be selected by the Council and approved by the Society: two representatives on the Council of American Association for the Advancement of Science for one-year term; one trustee on the Tropical Plant Research Foundation for a five-year term; and, one member of the Editorial Board of the American Journal of Botany for a three-year term.

AMENDMENTS

11. These rules may be amended by a majority vote of the members voting at any regular meeting of the Society.

A CORRECTION

In the paper by Kenneth W. Cooper, entitled "Relations of *Pediculopsis graminum* and *Fusarium poae* to central bud rot of carnations," *Phytopath.* 30: 853-859, the center heading, Description of Stewart's Bud Rot, should read, Description of Central Bud Rot. Also, paragraph 1, line 1, under center heading, Distribution of Central Bud Rot, change Stewart's to Central.

EDWARD JACOB PETRY
1880-1939

ERNST A. BESSEY

I first met Dr. Edward J. Petry about 20 years ago when he was an instructor in Botany at the University of Michigan, but it was not until 1924 when he came to Michigan State College as a candidate for the Ph.D. degree that I learned to know him well. He was the first graduate student to earn this degree in residence at that institution. He was the outstanding candidate out of the many in whose examinations I have participated, who was able to give a quick and completely satisfactory answer to every question put to him, not merely in the field of his thesis but in the whole field of



EDWARD JACOB PETRY

botany, as well as of bacteriology, his chief minor subject. It was a most brilliant performance. The thesis was a study of the root-nodules of *Ceanothus*, both from the bacteriological and from the plant physiological aspects. Although a work of great merit, recording great technical ingenuity as well as research ability, this was never published.

Dr. Petry was a native of Ohio, having been born near Gnadenhutten, O., June 24, 1880. His family was unable to give him schooling beyond the grades so that he was forced to earn his way through high school and uni-

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versity, in part, as a grade-school teacher, as a railroad fireman, and as a machinist in a machine shop. He was graduated from Ohio State University in 1907 and then became an assistant in botany at Cornell University for three years. Then, until 1918, he was connected with the Agronomy and Botany Departments of Purdue University, receiving his Master of Science degree at that institution in 1914. From 1918 to 1920 he was an instructor in Botany at the University of Michigan. Then, till he entered Michigan State College as a graduate student, he was Professor of Botany at South Dakota State College. After receiving his Ph.D. degree in 1925, he was survey botanist for the South Dakota Geological and Biological Survey, 1925-26. He re-entered the teaching profession and taught in Hendrix College, Conway, Ark.; Central College, Fayette, Mo.; and Coe College, Cedar Rapids, Iowa, from 1926 to 1933. This was the beginning of the depression times when denominational colleges were retrenching, so that finally, in 1933, he had to go into chemical and biological research for city and industrial plants. At the time of his death, October 8, 1939, he was Paint Chemist for the Ebony Paint Company, Kansas City, Mo., and Professor of Chemistry and Medical Technologist at Central College of Osteopathy in the same city.

Dr. Petry's interests in botany lay chiefly in plant pathology and plant physiology. He was a life member of the American Phytopathological Society and was at other times a member of several other scientific societies. When I knew him I was amazed at his knowledge of botanical literature, a matter of remark in consideration of the fact that the last decade of his life he was located away from the larger university centers.

He was a man of deep religious conviction and of sterling character. His scientific rectitude was such that he was very critical of poor, careless, or dishonest work, and this often brought him into conflict with others. I have met some of his students, and he had passed on to them an enthusiasm for good work. In his passing our society has lost an able member.

LIST OF PUBLICATIONS

1. Nutrients in green shoots of trees. Proc. Ind. Acad. Sci. 1911: 321-324. 1912.
2. Correlation of variation of resin content of *Podophyllum* with certain habitats. (With W. R. M. Scott, senior author.) Mich. Acad. Sci. Ann. Rept. 21 (1919): 225-231. 1920.
3. Germination and growth of *Ceanothus americanus* as affected by heated soils. Mich. Acad. Sci. Ann. Rept. 22 (1920): 135-142. 1921.
4. Some phases of plant feeding and plant diseases. S. Dak. State Hort. Soc. Ann. Rept. 18: 38-42. 1921.
5. Rhizoctonia, Corticium, or scurf, a serious potato disease in South Dakota. Proc. S. Dak. Acad. Sci. 6: 82-84. 1922.
6. Certified potato seed inspection. S. Dak. State Hort. Soc. Ann. Rept. 19: 45-47. 1922.
7. Conjugation in the aecium of *Dicraoma distichlidis*. (With L. D. Hutton, junior author.) Phytopath. 14: 33-34. (Abstract) 1924.
8. Structural modifications in *Bryophyllum calycinum*. Proc. S. Dak. Acad. Sci. 8: 22-23. 1924.
9. New plants of South Dakota. Proc. S. Dak. Acad. Sci. 8: 24. 1924.
10. A new potato disease in South Dakota. Proc. S. Dak. Acad. Sci. 8: 28-30. 1924.
11. The crown gall situation. S. Dak. State Hort. Soc. Ann. Rept. 21: 93-98. 1924.

12. Orchard sanitation. S. Dak. State Hort. Soc. Ann. Rept. 21: 105-109. 1924.
13. Plant treasures of the Black Hills. S. Dak. State Hort. Soc. Ann. Rept. 21: 122-133. 1924.
14. Weeds and their control. S. Dak. Agr. Exp. Stat. Bull. 211. 84 pp. 1924.
15. South Dakota. (With S. S. Visher, junior author.) In Shelford, V. E. Naturalists' Guide to the Americas. pp. 549-556. 1926.
16. The composition of cherry gum. Proc. S. Dak. Acad. Sci. 10: 18-24. 1927.
17. Additions to the South Dakota flora. Proc. S. Dak. Acad. Sci. 10: 25-27. 1927.
18. Some problems in the physiology of *Ceanothus* species. Proc. S. Dak. Acad. Sci. 10: 28-34. 1927.
19. Addition to the flora of Linn County. Proc. Iowa Acad. Sci. 40: 79. (Abstract) 1933.
20. Observations on the staining of bacterial flagella. Proc. Iowa Acad. Sci. 40: 79. (Abstract) 1933.
21. A new reservation area in Iowa. Proc. Iowa Acad. Sci. 40: 80. (Abstract) 1933.
22. The biota of the Cedar River as related to odor and taste production. Proc. Iowa Acad. Sci. 43: 123-126. 1936.

HOST SPECIALIZATION IN THE HEAD SMUT OF GRASSES, *USTILAGO BULLATA*¹

GEORGE W. FISCHER

(Accepted for publication June 3, 1940)

INTRODUCTION

Ustilago bullata Berk., causing head smut of various grasses, is one of the most composite smut species known.² Many species of the genera *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum*, and *Sitanion* are recognized as hosts (4, 5). This disease is known from the United States and Canada (especially the western regions), South America, New Zealand, Australia, Asia Minor, and Europe. In some localities it assumes destructive proportions on certain forage grasses. McAlpine (11) reported heavy infestation of head smut in prairie grass (*Bromus catharticus*)³ in Australia, and, according to Cunningham (3), this same species is sometimes heavily smutted in New Zealand. Head smut in slender wheatgrass (*Agropyron pauciflorum*) has been reported from western Canada (6, 13) in alarming proportions, and to a lesser degree from the western United States (4), and from western Siberia (12). In the western United States grasses of the "mountain brome" group (*Bromus carinatus* and allies) are commonly observed affected with head smut. This applies especially to *B. marginatus* Nees. and *B. polyanthus*, native stands of which are commonly smutted. In nursery

¹ Grass-disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Soil Conservation Service, Division of Conservation Nurseries, U. S. Department of Agriculture, and the Divisions of Plant Pathology and Agronomy of the Washington Agricultural Experiment Station, published as Scientific Paper No. 456, College of Agriculture and Agricultural Experiment Station, State College of Washington.

² In this paper the binomial *Ustilago bullata* is used in the sense that was recommended by the writer (4) in 1937 to include, on the basis of comparative morphology, *U. bromivora* (Tul.) Fisch. v. Waldh. and *U. lorentziana* v. Thüm. thought to be specialized to *Bromus* spp. and *Hordeum* spp., respectively.

³ Authorities for all grass species and varieties mentioned are given in the list appended below, under "Materials and Methods." The writer is indebted to Mr. J. R. Swallen for checking the nomenclature, especially of introduced species. Hitchcock (9) has been followed for nomenclature of native species.

rows the last-named species and *B. catharticus* often show 40-60 per cent natural infection, and sometimes even more.

Certain other species often exhibit even greater percentages of head smut in native stands than are manifest by the more strictly forage species such as are mentioned above. Thus, in certain sections of the Pacific Northwest, especially the "waste" land type, vast areas occur in which *Bromus tectorum* (cheatgrass, downy cheat, downy chess) is the predominant species, with 10 to almost 100 per cent of the plants affected with head smut. *B. tectorum* also is common as a weed in the more productive areas of the intermountain region of the Pacific Northwest. Here also, head smut is so common that smut-free stands are comparatively rare and areas containing high percentages of smut are legion. The smut also is quite prevalent on *B. mollis* (soft chess) in the Pacific Northwest, but the percentages of infection are usually lower than on *B. tectorum* and the distribution is less general.

In addition to those named, *Ustilago bullata* occurs on some 40 other grass species in the West, so that it probably is the most common smut in this region of the United States.

The regular occurrence of head smut in epiphytotic form on certain common and widespread native grasses and the less common occurrence on numerous other species, both native and introduced, has fostered some interest in the probabilities of host specialization and the existence of physiologic races in *Ustilago bullata*. Certain field observations suggested some degree of host specialization in head smut on *Bromus* spp. For example, in 1934-35, the writer observed that, although head smut was more or less prevalent on *B. tectorum*, *B. mollis*, and *B. marginatus* in the vicinity of Pullman, Washington, it was not co-extensive on these hosts. Frequently, stands of mixed *B. tectorum* and *B. marginatus* were found wherein the former was heavily smutted and the latter smut-free. Similar observations were made with regard to mixed stands of *B. mollis* and *B. tectorum*, and, consequently, it began to appear probable that each of the 3 grasses was harboring a different race of the head-smut organism.

Therefore, cross-inoculation experiments were begun in 1935 to determine whether there are physiologic races of *Ustilago bullata*, and also to determine the extent to which the common native grasses which regularly are heavily smutted can serve as a source of infection of the more valuable forage species. The investigations were begun with 4 collections of *U. bullata*, and this number has increased yearly to the present number of 77.

REVIEW OF LITERATURE

The literature relating to host specialization and physiologic races in *Ustilago bullata* is very meager; the most of what little has been published is the work of Liro (10) in Finland. He began by inoculating seed of *Bromus secalinus*, *B. mollis*, *B. arvensis*, and *B. erectus* with head smut from *B. secalinus*, with the result of no smut on any but the latter species. Similar additional experiments proved that *B. secalinus*, *B. arvensis* and *B. mollis* each

harbors its own race of head smut. On the basis of such host specialization Liro recognized the smut on *B. secalinus* as the type of *U. bromivora* (here included in *U. bullata*, see footnote 2), and gave specific designation to the other two. The smut on *B. arvensis* became *U. bromi-arvensis* Liro, n. sp., and that on *B. mollis*, *U. bromi-mollis* Liro, n. sp., in spite of the fact that Liro fully recognized that the new "species" were indistinguishable from *U. bromivora* on any morphologic basis.

In Canada, Fraser and Scott (6) in a preliminary investigation of head smut in "western ryegrass" (slender wheatgrass, *Agropyron pauciflorum*) inoculated *A. pauciflorum*, *A. subsecundum*, *A. dasystachyum*, *Bromus ciliatus*, and *B. latiglumis* with spores from *A. pauciflorum*. The percentage of smut was rather high on *A. pauciflorum* (50 per cent) but very low on the other species inoculated. This was the first step in determining the host range of the head smut in slender wheatgrass, which was shown to include *A. subsecundum*, *A. dasystachyum*, *B. ciliatus*, and *B. latiglumis*, with some doubt, however, since no check rows were included and the percentages of infection were low. Later, Padwick and Henry (13) inoculated these and other economic species of *Agropyron* with head smut from *A. pauciflorum*, and obtained infection on *A. dasystachyum*, *A. griffithsi* and *A. subsecundum*. Again, the percentages of infection were low (9-26 per cent) as compared with 100 per cent on *A. pauciflorum*, and no check rows were included.

MATERIALS AND METHODS

During the years 1934-39 many collections of *Ustilago bullata* from various grasses have been made by the writer, and others have been received from correspondents. Each collection was given a symbol to indicate to some extent its host origin. Thus, collections from *Bromus* spp. were designated with the letter "M," those from *Hordeum* and *Sitanion* spp. the letter "R," and from *Agropyron*, *Elymus*, and *Festuca* the letter "N," as explained in footnote a, table 1. Of the scores of collections of head smut made by the writer and collaborators, 44 are here reported. These have been selected on the basis of host species, although occasionally more than one collection was kept from the same host species. These 44 collections, together with collection data, are listed in table 1.

Most of the inoculations were performed by Zade's partial vacuum method, as described by Allison (1). Besides contributing generally toward higher percentages of infection, the method has the additional advantage of minimizing chances of contamination from air-borne spores.

In some instances the spore material in a collection was too meager to allow for extensive inoculations. In such instances monosporidial cultures of opposite sex were secured and multiplied on nutrient agar to a quantity sufficient for inoculations. The inoculation methods when sporidia were used instead of spores also varied. It was found quite satisfactory at first to germinate the seeds of the desired species, mix (on plain agar) masses of sporidia of opposite sex representing the collection, and then, after the

TABLE 1.—Collection data concerning 44 collections of *Ustilago bullata* under investigation at Pullman, Washington

Col. Sym-bol	Host	Locality	Date	Collector
M-A ^a	<i>Bromus tectorum</i>	Pullman, Wash.	June, 1935	G. W. Fischer
M-B	" <i>mollis</i>	" "	7/6/35	"
M-C	" <i>marginatus</i>	" "	7/5/35	"
M-E	" <i>squarrosus</i>	" " SCN ^b	July, 1936	"
M-F	" <i>japonicus</i>	Manhattan, Kansas	June, 1935	C. L. Lefebvre
M-G	" <i>erectus</i>	Pullman, Wash. SCN	July, 1936	G. W. Fischer
M-H	" sp.	Silver Plume, Colo.	1936	C. O. Johnston
M-I	" <i>catharticus</i>	Astoria, Oregon	Aug., 1936	L. A. Mullen
M-J	" <i>squarrosus</i>	Pullman, Wash. SCN	July, 1936	G. W. Fischer
M-K	" sp.	" " "	"
M-L	" <i>anomalus</i>	W. Yellowstone, Mont.	8/7/36	L. P. Reitz
M-N	" <i>sterilis</i>	Pullman, Wash.	June, 1935	G. W. Fischer
M-O	" <i>brizaeiformis</i>	Graingeville, Ida.	1936	Unknown
M-P	" <i>secalinus</i>	Creswell, Oregon	8/10/37	G. W. Fischer
M-Q	" <i>inermis</i>	Pullman, Wash. SCN	8/19/37	"
M-R	" <i>catharticus</i>	Waikii, Hawaii	June, 1937	K. F. Baker
M-S	" <i>rubens</i>	Coalinga, Calif.	5/ 5/38	C. A. Suneson
M-T	" sp.	Salina, Calif.	" "	"
M-U	" <i>macrostachys</i>	Pullman, BPI Nurs.	7/ 1/38	G. W. Fischer
M-V	" <i>purgans</i>	Pullman, Wash. SCN	6/24/38	"
M-W	" <i>polyanthus</i>	" " "	7/ 2/38	"
M-X	" sp.	" " "	" "	"
M-Y	" <i>inermis</i>	Bozeman, Mont. BPI Nurs.	Aug., 1938	L. P. Reitz
N-A	<i>Agropyron pauciflorum</i>	Pullman, Wash. SCN	7/2/36	G. W. Fischer
N-B	" <i>cristatum</i>	Lind, Wash., Exp. Sta. Nurs.	June, 1936	"
N-C	" <i>dasy-stachyum</i>	Pullman, Wash. SCN	July, 1936	"
N-D	" <i>scabrum</i>	Australia (Herb. spec.)	C. C. Brittlebank
N-E	" <i>inermis</i>	Pullman, Wash. SCN	6/30/37	G. W. Fischer
N-F	<i>Elymus sibiricus</i>	" " "	7/29/37	"
N-G	" <i>glaucus</i>	" " "	8/20/37	"
N-I	" <i>sibiricus</i>	" " "	July, 1937	"
N-J	" <i>canadensis</i>	" " "	8/21/37	"
N-K	" "	" " "	Aug., 1937	"
N-L	<i>Festuca idahoensis</i>	" " "	6/15/38	"
N-M	<i>Elymus junceus</i>	" " "	6/29/38	D. C. Smith
N-N	<i>Festuca idahoensis</i>	" " "	6/29/38	G. W. Fischer
N-O	<i>Agropyron smithii</i>	" " "	7/ 2/38	"
N-P	" <i>dasy-stachyum</i>	" " "	" "	"
N-Q	" <i>cantium</i>	" " "	7/ 1/38	"
N-R	" <i>orientale</i> var. <i>lasianthum</i>	" " "	July, 1938	C. Riesenweber
N-S	<i>Elymus canadensis</i>	Bozeman, Mont. BPI Nurs.	Aug., 1938	L. P. Reitz
R-A	<i>Hordeum nodosum</i>	Pullman, Wash. SCN	6/10/35	G. W. Fischer
R-E	" <i>gussonianum</i>	Manhattan, Kans. via Davis, California	1936	C. L. Lefebvre
R-G	<i>Sitanion jubatum</i>	Weed, California	6/6/37	C. A. Suneson

^a The first letter in each case indicates the generic group, M=*Bromus*; N=*Agropyron*, *Elymus*, and *Festuca* spp.; R=*Hordeum* and *Sitanion* spp. The second letter indicates the species.

^b SCN refers to the fact that the collection was made in the observational nurseries or in the increase plots of the Pullman, Washington, unit of the Soil Conservation Service, Division of Nurseries.

sporidia had fused, to place the slightly-germinated seeds in contact with the sporidia. With most grass seeds better results were obtained by removing the lemma, so as to place the scarcely developed coleoptile in direct contact with the infection hyphae arising from the fused sporidia.⁴ After the seed had germinated to a height of the Petri-dish cover, seed and agar were placed in pots of soil. While 100 per cent infection commonly resulted from this method, it became too laborious when the work had progressed to the point where a dozen or more collections of head smut were in use. The partial-vacuum method was then resorted to, using suspensions of sporidia of opposite sex, as with chlamydospores, and high percentages of infection were obtained by this method also.

The inoculated seed usually was sown directly in nursery rows, although sometimes it was seeded in pots in the greenhouse, and later transplanted to the field. This latter method was not feasible for large numbers. When the inoculated seed was sown directly in nursery rows, certain problems were encountered. Too often fall-seeding resulted in very poor stands due to winter-killing in some cases, and/or frost heaving in others. Spring-sown seed of many species would fail to head the first season. With these difficulties to be faced, a method was devised whereby good stands, high percentages of infection, and maturation the first season were better assured. The method consisted in planting the inoculated seed, while still wet (the labor of preliminary drying after vacuum inoculation is also eliminated), in pots or plant bands of soil in the greenhouse. When the seedlings appeared above the soil they were covered with granulated peat moss or pulverized, well-rotted stable manure and removed in the pots or bands to a cold frame for the winter. The seed usually was started in November or December, allowed to remain in the cold frame during the winter, and then transplanted to the field in the spring as early as the ground could be prepared. This method has the effect of vernalization on the young grass seedlings, so that they grow vigorously after transplantation to the field and head out well.

All seed lots used in these cross-inoculation experiments were first treated with 1-320 formaldehyde solution for 30 min. to 1½ hr., depending on specific toleration to formaldehyde and the length of time required for sterilization, as determined by trial beforehand. The treated seed was always thoroughly washed to remove the formaldehyde and then dried before using.

In order to obviate the necessity of giving authorities elsewhere in this paper for all the grass species and varieties mentioned, they are listed in one group as follows:

Agropyron caninum (L.) Beauv., *A. cristatum* (L.) Gaertn., *A. dasystachyum* (Hook.) Scribn., *A. griffithsi* Scribn. & Smith, *A. inerme* (Scribn. & Smith) Rydb., *A. orientale* var. *lasianthum* (Boiss.) Boiss., *A. pauciflorum* (Schwein.) Hitchc., *A. repens* (L.) Beauv., *A. scabrum* (Labill.) Beauv., *A. semicostatum* (Steud.) Nees, *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb.,

⁴ This method refers to unpublished life-history studies to be reported in a later paper.

A. spicatum (Pursh) Scribn. & Smith, *A. subsecundum* (Link) Hitchc., *A. trichophorum* (Link) Richt.; *Bromus anomalus* Rupr., *B. arduennensis* Dum., *B. arvensis* L., *B. brachystachys* Hornung, *B. brevis* (Nees) Steud., *B. brizaeformis* Fisch. & Mey., *B. carinatus* Hook. & Arn., *B. catharticus* Vahl, *B. ciliatus* L., *B. commutatus* Schrad., *B. erectus* Huds., *B. hordeaceus* L., *B. inermis* Leyss., *B. japonicus* Thunb., *B. kalmii* A. Gray, *B. lanuginosus* Poir., *B. latiglumis* (Shear) Hitchc., *B. macrostachys* Desf., *B. madritensis* L., *B. marginatus* Nees, *B. mollis* L., *B. pacificus* Shear, *B. polyanthus* Scribn., *B. purgans* L., *B. rigidus* Roth, *B. rigidus gussonii* (Parl.) Goss. & Dur., *B. rubens* L., *B. secalinus* L., *B. squarrosus* L., *B. sterilis* L., *B. tectorum* L., *B. vulgaris* (Hook.) Shear; *Elymus canadensis* L., *E. glaucus* Buckl., *E. junceus* Fisch., *E. sibiricus* L., *E. villosus* Muhl., *E. virginicus* L.; *Festuca idahoensis* Elmer; *Hordeum brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. gussonianum* Parl., *H. jubatum* L., *H. jubatum* var. *caespitosum* (Scribn.) Hitchc., *H. murinum* L., *H. nodosum* L.; *Sitanion hystrix* (Nutt.) J. G. Smith, *S. jubatum* J. G. Smith.

RESULTS

1936 Inoculation Experiments

Collections R-A, R-B, and R-C of *Ustilago bullata*, from *Hordeum nodosum* and *H. jubatum*, were used to inoculate *Elymus villosus*, *Hordeum murinum*, *H. gussonianum*, *H. nodosum* and *H. jubatum*. All 3 collections produced 75-100 per cent smut on *H. nodosum* and *H. jubatum*, but none on

TABLE 2.—Results of inoculations of 20 *Bromus* spp. with 4 collections of *Ustilago bullata* in 1936

Species	F No. ^a	Source of Inoculum			
		<i>B. tectorum</i>	<i>B. mollis</i>	<i>B. marginatus</i>	<i>B. polyanthus</i>
		Symbol			
		M-A	M-B	M-C	M-D
		Smut ^b	Smut	Smut	Smut
		Per cent	Per cent	Per cent	Per cent
<i>B. arduennensis</i>	161	0	100	100
" <i>brachystachys</i>	160	0	100	0	0
" <i>commutatus</i>	157	0	33.3	0	0
" <i>japonicus</i>	154	100	0	0	0
" <i>marginatus</i>	79	0	0	57.1	66.6
" <i>mollis</i>	168	0	100	0	0
" <i>polyanthus</i>	51	0	0	75	100
" <i>catharticus</i>	145	0	88.8	66.6
" <i>sterilis</i>	144	100	0	0	0
" <i>tectorum</i>	142	100	0	0	0
" <i>catharticus</i>	141	0	0	100	100
Other species ^c		0	0	0	0

^a Writer's accession number.

^b On plant basis.

^c In this group are *Bromus commutatus* (F 167), *B. gussonii* (F 156), *B. hordeaceus* (F 155), *B. inermis* (F 52 and 80), *B. kalmii* (F 153), *B. lanuginosus* (F 152), *B. macrostachys* (F 151), *B. madritensis* (F 150), *B. rigidus* (F 147), and *B. rubens* (F 146).

the other species except for 1 smutted plant of *E. villosus* inoculated with R-B. Collections R-A, R-B, and R-C thus appeared to be of similar pathogenicity.

Twenty *Bromus* spp. were inoculated with collections M-A, M-B, M-C, and M-D from *Bromus tectorum*, *B. mollis*, *B. marginatus*, and *B. polyanthus*, respectively. The results of these inoculations, recorded in table 2, indicated that collections M-A, M-B, M-C, and M-D represent 3 very distinct races of *Ustilago bullata*, M-C and M-D appearing identical.

1937 Inoculation Experiments

Field collections of *Ustilago bullata* during 1936 increased the number to be used for cross-inoculation experiments in 1937 to 19, of which number 12 were from *Bromus* spp., 4 from *Agropyron* spp., and 3 from *Hordeum* spp.

Eighteen accessions of species of *Agropyron*, *Elymus*, *Hordeum*, and *Sitanion* were inoculated each with some or all of the 7 collections of *Ustilago bullata* on *Agropyron* and *Hordeum*. The infection results are given in table 3, the recorded percentage being from the duplicate inoculation which resulted in the more smut.

TABLE 3.—The reaction of *Agropyron*, *Elymus*, *Hordeum*, and *Sitanion* spp. to 7 collections of *Ustilago bullata* from *Agropyron* and *Hordeum* spp. in 1937

Species	Writer's Acc. No.	Other No.	<i>A. pauciflorum</i>	<i>A. cristatum</i>	<i>A. dasystachyum</i>	<i>A. scabrum</i>	<i>H. nodosum</i>	<i>H. jubatum</i>	<i>H. gussoneanum</i>
			N-A	N-B	N-C	N-D	R-A	R-B	R-E
			Highest percentage infection obtained						
<i>A. pauciflorum</i>	67		100	0	0	0	85	33	50
" "	56	Wn. 279 ^c	n. s. ^a	0	0	0	75	100	0
" "	73	Wn. 434	15	0	0	0	100	14	0
" "	84		73	0	0	20	25	0	20
<i>A. caninum</i>	282	W. 17	0	20	0	0	50	67	0
<i>A. spicatum</i>	264	W. 740	50	0	29	0	0	0	n. s. ^a
<i>E. glaucus</i>	4		14	0	0	0	33	40	16
<i>H. brevisubulatum</i>	302	W. 303	75	0	0	0	100	83	13
<i>H. nodosum</i>	274	W. 2723	100	0	0	13	100	100	33
<i>S. hystrix</i>	278		n. s. ^a	0	— ^b	— ^b	100	100	0
Other species ^d			0	0	0	0	0	0	0

^a n. s. = no stand

^b — = not inoculated

^c W. numbers are accession numbers of the Pullman unit of the Soil Conservation Service, Division of Nurseries; Wn. numbers are those of the Washington Agricultural Experiment Station.

^d In this group are *Agropyron cristatum* (F 290, 304, 314), *A. repens* (F 140, 297), *Hordeum bulbosum* (F 303), *H. gussoneanum* (F 116), and *H. nodosum* (F 122).

Each of 38 accessions representing 24 species of *Bromus* were inoculated with 12 individual collections of *Ustilago bullata* from *Bromus* spp. in the same manner as with the inoculations of *Agropyron*, *Elymus*, *Hordeum*, and *Sitanion* just described. The infection data are recorded in table 4, giving the higher percentage of smut resulting from duplicate inoculations.

TABLE 4.—Reaction of 33 collections of *Bromus* spp. to 12 collections of *Ustilago bullata* from *Bromus* spp. in 1937

Species	Writer's Acc. No.	Other No.	Highest percentage infection obtained											
			<i>B. tectorum</i> M-A	<i>B. mollis</i> M-B	<i>B. marginatus</i> M-C	<i>B. squarrosus</i> M-E	<i>B. japonicus</i> M-F	<i>B. erectus</i> M-G	<i>B. catharticus</i> M-I	<i>B. squarrosus</i> M-J	<i>Bromus</i> sp. M-K	<i>B. anomalus</i> M-L	<i>B. sterilis</i> M-N	<i>B. brizaeformis</i> M-O
<i>B. brachystachys</i>	160	0	60	0	0	45	0	0	0	0	0	0	0
<i>B. brevis</i>	319	0	0	0	0	0	0	50	0	0	67	0	0
<i>B. carinatus</i>	186	0	0	12	0	0	0	0	0	0	0	0	0
“	239	W. 2725 ^a	0	0	50	0	0	0	0	0	0	0	0	0
<i>B. catharticus</i>	145	0	0	86	0	0	0	100	0	0	100	0	0
“	161	0	0	100	0	0	0	100	0	0	70	0	0
<i>B. comatus</i>	157	0	50	0	0	0	0	0	0	0	0	0	15
<i>B. erectus</i>	246	W. 1727	33	0	0	0	0	33	0	0	33	0	0	0
“	316	38	0	0	0	0	0	0	0	0	0	0	0
<i>B. hordeaceus</i>	155	0	25	0	0	0	0	0	0	0	0	0	20
<i>B. inermis</i>	236	W. 1739	35	0	0	0	0	0	0	0	0	0	0	0
“	238	W. 1734	20	0	0	0	0	0	0	0	0	0	0	0
<i>B. japonicus</i>	154	0	0	0	33	50	0	0	0	0	0	0	0
<i>B. macrostachys</i>	151	0	20	0	0	0	0	0	0	0	0	0	0
<i>B. marginatus</i>	48	Wn. 439	0	0	0	0	0	0	0	0	0	0	0	0
“	241	W. 1842	0	0	80	0	0	0	50	0	0	0	0	0
“	242	W. 2780	0	0	40	0	0	0	10	0	0	0	0	0
“	243	W. 2133	0	0	25	0	0	0	0	0	0	0	0	0
“	194	0	40	0	0	0	0	0	0	0	0	0	0
<i>B. mollis</i>	184	0	33	0	0	0	0	0	0	0	0	0	22
<i>B. pacificus</i>	50	Wn. 437	0	0	95	0	0	0	0	0	0	0	0	0
<i>B. polyanthus</i>	143	0	0	0	100	0	0	0	86	0	0	0	20
<i>B. squarrosus</i>	276	0	0	0	20	0	0	0	71	0	0	90	0
“	165	W. 182	100	0	0	0	0	0	0	0	100	0	0	0
<i>B. tectorum</i>	244	0	0	0	0	0	0	100	0	0	0	0	0
<i>B. vulgaris</i>	283	W. 2640	0	0	0	0	0	0	0	0	100	0	0	0
<i>Bromus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
Other species ^b	0	0	0	0	0	0	0	0	0	0	0	0

^a W. numbers are those of the Pullman unit of the Soil Conservation Service, Division of Nurseries; Wn. numbers are those of the Washington Agricultural Experiment Station.

^b In this group are *Bromus carinatus* (F 280), *B. inermis* (F 52, 80), *B. kalmii* (F 153), *B. lanuginosus* (F 152), *B. madritensis* (F 150), *B. rigidus* (F 147, 275).

The data in tables 3 and 4 presented some interesting indications. The 19 collections of *Ustilago bullata* used in the 1937 experiments seemed to represent several well-defined races, each with a rather restricted host range. Except for some slight differences, collections N-A, R-A, R-B, and R-E seemed to have similar pathogenicity, being virulent on *Agropyron pauciflorum*, *Elymus canadensis*, *Hordeum brevisubulatum*, *H. nodosum*, and *Sitanion jubatum*. Collections N-B and N-C appeared to be much less virulent, not seeming to have truly compatible hosts in any of the species inoculated, except for 83 per cent infection of *E. canadensis* with N-B, and here the type of infection was such that the sori were poorly developed. Even though N-B was from *A. cristatum*, it failed to produce smut on this species. Obviously these two collections merited further study, using other grass species and genera. Collection N-D also appeared quite distinct. Only *A. pauciflorum* and *H. nodosum* showed any susceptibility; even here the percentages of infection were low, although the sori were large and well-developed. Collections M-A and M-N from *Bromus tectorum* and *B. sterilis* appeared to comprise a distinct race of *U. bullata* with *B. tectorum* being the most susceptible grass of those inoculated, although *B. erectus* and *B. inermis* showed some infection from M-A but none from M-N. Similarly, collections M-B and M-O, from *B. mollis* and *B. brizaeformis*, respectively, seemed similar, but it was recognized that they would have to be studied further. The collections M-I and M-L appeared related to each other and somewhat also to M-C by their virulence on *B. catharticus*, yet M-C appeared to be much more virulent on *B. marginatus* and *B. polyanthus* than did the other two collections. Collection M-F, from *B. japonicus*, appeared distinct, producing infection on *B. brachystachys* and *B. japonicus* only. Finally, collection M-K, from *Bromus* sp., likewise appeared distinct from the other collections, with only *B. erectus* showing infection.

1938 Inoculation Experiments

Twenty-seven collections of *Ustilago bullata* from various grasses were tested. These included the head-smut collections made in the field in 1937. The inoculation experiments of 1938 were carried out with a different viewpoint than characterized those of the 1936 and 1937 seasons. The preliminary cross-inoculation experiments of limited extent of 1935-36 had indicated that collections or races of *Ustilago bullata* from *Agropyron* and *Hordeum* are restricted to those genera, and collections or races from *Bromus* spp. are restricted to *Bromus* spp. Consequently, in later experiments inoculations of *Agropyron*, *Elymus*, and *Hordeum* were made with collections of smut from grasses of those genera only, and inoculations of *Bromus* spp. were made only with collections from *Bromus* spp. By 1938, however, the writer began to suspect that the possibilities of *Bromus* races of *U. bullata* being able to infect other genera, and of *Agropyron*, *Elymus*, and *Hordeum* races to infect *Bromus* spp. merited a more thorough investigation than had been provided in the earlier experiments. The following observations con-

tributed toward this conviction: 1. Certain collections of head smut, e.g., N-B from *Agropyron cristatum* and N-C from *A. dasystachyum*, when inoculated on to *Agropyron* and *Hordeum* spp. (where it was assumed they belonged), behaved in a manner suggesting that possibly they might have originated from a grass not related to these genera. Even by inoculating several accessions of *A. cristatum* with N-B, from *A. cristatum*, no trace of infection resulted, which suggested that crested wheatgrass is not the real host to this collection of *U. bullata*. Inoculation experiments, using collection N-C from *A. dasystachyum* gave similar results. 2. Studies of the spore morphology, spore germination, and cultural characters⁵ of all the collections of *U. bullata*, including those made during 1937, very strongly suggested a greater relationship of certain of the collections of head smut from *Agropyron* and *Elymus* spp. to certain collections from *Bromus* spp. than they had to other collections from *Agropyron* and *Elymus* spp.

With this in mind a rather extensive cross-inoculation experiment was carried out in 1938. From the many accessions and species of grasses inoculated in previous years, and from certain accessions on which head smut was collected in 1938, 21 species of *Agropyron*, *Bromus*, *Elymus*, and *Hordeum* were selected as a step toward the establishment of differential species or "host-testers" for races of *Ustilago bullata*. Each of these was inoculated with each of the 27 selected collections of head smut from *Agropyron*, *Bromus*, *Elymus*, and *Hordeum* spp. Complete cross-inoculation was effected in duplicate, with chlamydospores as inoculum in one case, and suspensions of sporidia of opposite sex as inoculum in the other. High percentages of infection resulted from both inoculations.

The infection data from these 1938 inoculations are presented in table 5 in which the higher percentage of infection from the duplicate inoculations is recorded; no attempt was made to compare in detail the results of the 2 methods. From the results in table 5 it is seen that the supposed specialization of *Ustilago bullata* from brome-grasses to brome-grasses, and from grasses of the *Hordeae* to that group, does not hold. Obviously, therefore, the previous inoculation experiments were not sufficiently extensive, besides not including certain collections which in the 1938 experiments proved capable of such cross-infection. The results of the 1938 inoculations show why collections N-B and N-C from *Agropyron cristatum* and *A. dasystachyum*, respectively, were, in the earlier experiments, unable to infect these and other species of *Agropyron*. The evidence indicates that collections N-B, N-C, N-E, and N-G represent the infection under natural conditions of *Agropyron cristatum*, *A. dasystachyum*, *A. inerme* and *Elymus glaucus*, respectively, with head smut from the very common and widespread weed, *Bromus tectorum*. Collection N-K represents similar infection of *E. canadensis* with the head smut from *B. mollis*. As already mentioned, both *B. tectorum* and *B. mollis* are common grasses in the Pacific Northwest and the smut on these is essentially co-extensive with their distribution in this territory. These

⁵ To be included in a later paper.

TABLE 5.—Summary of 1938 inoculations of *Agropyron*, *Bromus*, *Elymus*, and *Hordeum* spp. with 27 collections of *Ustilago bullata*

Differential species	Writer's Acc. No.	Source and designation of the collections														
		<i>A. pauciflorum</i>	<i>A. cristatum</i>	<i>A. dasystachyum</i>	<i>A. scabrum</i>	<i>A. inermis</i>	<i>E. sibiricus</i>	<i>E. glaucus</i>	<i>E. canadensis</i>	<i>E. canadensis</i>	<i>H. nodosum</i>	<i>H. gussoneanum</i>	<i>S. hystrix</i>	<i>B. tectorum</i>	<i>B. mollis</i>	
<i>A. caninum</i>	282	R ^a	R	R	R	R	S25 ^d	R	R	s8	R	R	R	R	
"	138	Sb100	R	s10	S35	R	S25	R	R	R	S90	S90	S25	R	R	
<i>A. pauciflorum</i>	67	S34	R	R	R	R	S100	R	R	S67	S100	S10	S80	R	R	
<i>E. canadensis</i>	341	S100	S ^c 6	R	R	R	S100	s65	R	S100	S100	S100	S100	R	R	
" <i>glaucus</i>	342	S20	R	S75	R	S10	S25	S25	R	R	S50	R	R	R	R	
" <i>sibiricus</i>	338	S100	S20	S15	R	S50	S75	s50	R	S100	S60	S90	S60	s15	S15	
<i>H. nodosum</i>	169	S100	R	R	R	R	S100	s10	R	S100	S100	S100	S100	R	S20	
"	274	S100	S25	S28	R	s8	S100	R	R	S100	S100	S100	S100	R	S20	
<i>B. brizaeiformis</i>	245	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>carinatus</i>	239	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>erectus</i>	336	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>inermis</i>	238	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>japonicus</i>	154	R	R	S100	R	s100	R	S50	R	R	R	R	R	R	S15	
" <i>marginatus</i>	48	R	R	R	R	R	R	R	R	R	R	R	R	R	S50	
"	241	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>mollis</i>	194	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>catharticus</i>	145	R	R	R	R	R	R	R	R	R	R	R	R	R	S50	
" <i>secalinus</i>	329	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>squarrosus</i>	143	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
" <i>tectorum</i>	165	R	S50	R	R	R	R	S100	R	R	R	R	R	R	S100	

^a R = Highly resistant or immune.^b S = Truly susceptible as shown by well-developed normal sori.^c s = Susceptible but characterized by latent sori.^d Percentage of infection on plant basis.

1938 inoculations yielded some startling information concerning the host ranges of some of the other collections of *Ustilago bullata*, particularly those from *Bromus* spp. All of the collections of *U. bullata* from *Bromus* spp., except M-P, were found to be able to produce more or less infection on one or more of the members of the *Hordeae* included in the "differentials," showing very definitely that the races on *Bromus* spp. are not confined to members of that genus. Collections M-I and M-R especially seem to be able to produce smut as lavishly on certain *Agropyron*, *Elymus*, and *Hordeum* spp. as on their own host, *B. catharticus*.

These cross-inoculation experiments of 1938, so much more extensive than those of the preceding years, suggested the desirability of repetition as a check on the startling results of 1938. Accordingly, the entire experiment was repeated in the greenhouse during the fall and winter of 1938-39. Some of the species failed to come to head, so that the results are incomplete and hardly merit tabular presentation. It should be mentioned, however, that of those species that did head out the results checked with the field results of 1938. Nevertheless, it was decided that the 1939 experiments should include the same head-smut collections as were used in 1938.

1939 Inoculation Experiments

Collections of head smut in 1938 increased to 43 the number selected for cross-inoculation experiments in 1939. The number of hosts was reduced to 18, by eliminating those tending to give poor stands or duplicate reactions. Each of these "differential" species was inoculated with each collection of *U. bullata* shown in table 6. Excellent stands and high percentages of infection were obtained.

The results of the 1939 inoculations substantiate those of 1938, and provide further interesting information concerning the host range of certain races of *Ustilago bullata*. This is especially true of the race on cheatgrass, *Bromus tectorum*. These results indicate that there is a natural infection of *Agropyron cristatum*, *A. dasystachyum*, *A. inerme*, *A. caninum*, *Bromus erectus*, *B. inermis*, *Elymus glaucus*, *E. junceus*, *E. sibiricus*, and *Festuca idahoensis* from this common and widespread race on *B. tectorum*. This species again was included in the "differentials" in 1939, and, as seen in table 6, showed a high percentage of infection from 16 of the 43 collections of *U. bullata* used. Many of these instances of infection of *B. tectorum* by collections of head smut from a variety of other grasses has been obtained also in the 1938 field and greenhouse inoculations. A few others represent just one season's inoculations, but the results seem conclusive. These infections of *B. tectorum* with smut from other species are substantiated also by the fact that some of the other species have been infected with the collection M-A from *B. tectorum*. Others have not been tried. The virulence of collection M-B, from *Bromus mollis*, on *Elymus canadensis* and the virulence of collection N-K, from *E. canadensis*, on *B. mollis*, together with the fact that the two collections have similar host ranges, further demonstrates what

TABLE 6.—Summary of the reaction of species of *Agropyron*, *Bromus*, *Elymus*, and *Hordeum* to 43 collections of *Ustilago bullata* in 1939

Differential species	F No. ^a	Source and designation of the collections									
		<i>Agropyron pauciflorum</i>	<i>A. cristatum</i>	<i>A. dasystachyum</i>	<i>A. scabrum</i>	<i>A. inermis</i>	<i>Elymus sibiricus</i>	<i>B. glaucus</i>	<i>E. sibiricus</i>	<i>E. canadensis</i>	<i>E. canadensis</i>
<i>A. pauciflorum</i>	67	N-A	N-B	N-C	N-D	N-E	N-F	N-G	N-I	N-J	N-K
<i>B. brizaeformis</i>	245	S ^a 85 ^e	R	R	R	R	S88	R	R	S35	R
" <i>carinatus</i>	239	R ^b	R	R	R	R	R	R	R	R	R
" <i>catharticus</i>	161	R	R	R	R	R	R	R	R	R	R
" <i>erectus</i>	336	R	R	R	R	S50	R	R	R	R	R
" <i>hordeaceus</i>	155	R	R	R	R	R	R	R	R	R	S35
" <i>inermis</i>	385	R	R	R	R	R	R	R	R	R	R
" <i>japonicus</i>	154	R	R	R	R	R	R	R	R	R	R
" <i>marginatus</i>	48	R	R	R	R	R	R	R	R	R	S100
" <i>mollis</i>	194	R	R	R	R	R	R	R	R	R	R
" <i>rubens</i>	146	R	R	R	R	R	R	R	R	R	R
" <i>secalinus</i>	329	R	R	R	R	R	R	R	R	R	R
" <i>squarrosus</i>	143	R	R	R	R	R	R	R	R	R	R
" <i>tectorum</i>	165	R	S100	S100	R	S100	R	S100	R	R	S100
<i>E. canadensis</i>	341	S100	R	S85	S95	R	S100	R	S50	S75	R
" <i>glaucus</i>	342	S67	S100	S100	R	S100	S50	R	R	S100
" <i>sibiricus</i>	338	S100	R	R	R	S45	S100	S30	S50	S100	S25
<i>H. nodosum</i>	274	S100	R	R	S40	R	S100	R	R	S100	R

^a S = Truly susceptible as shown by well-developed normal sori.^b s = Susceptible but characterized by latent sori.^c R = Highly resistant or immune.^d F No. = Author's accession number.^e Percentage of infection on plant basis.

TABLE 6.—(Continued)

Differential species	F No. ^a	Source and designation of the collections										
		<i>Elymus junceus</i>	<i>F. idahoensis</i>	<i>A. smithii</i>	<i>A. dasystachyum</i>	<i>A. caninum</i>	<i>A. lasianthum</i>	<i>B. canadensis</i>	<i>Hordeum nodosum</i>	<i>H. gussonianum</i>	<i>Sitacion hystrix</i>	<i>Bromus tectorum</i>
		N-M	N-N	N-O	N-P	N-Q	N-R	N-S	R-A	R-E	R-G	M-A
<i>A. pauciflorum</i>	67	R	R	S60	R	R	R	S100	S60	S10	S	R
<i>B. brizaeformis</i>	245	R	R	R	R	R	R	R	R	R	R	R
“ <i>carinatus</i>	239	R	R	R	R	R	R	R	R	R	R	R
“ <i>catharticus</i>	161	R	R	R	R	R	R	R	R	R	R	R
“ <i>erectus</i>	336	R	R	R	R	R	R	R	R	R	R	R
“ <i>hordeaceus</i>	155	R	R	R	R	R	R	R	R	R	R	R
“ <i>inermis</i>	385	R	R	R	R	R	R	R	R	R	R	R
“ <i>japonicus</i>	154	S100	S50	R	R	R	R	R	R	R	R	R
“ <i>marginalis</i>	48	R	R	R	R	R	R	R	R	R	R	R
“ <i>mollis</i>	194	R	R	R	R	R	R	R	R	R	R	R
“ <i>rubens</i>	146	R	R	R	R	R	R	R	R	R	R	R
“ <i>secalinus</i>	329	R	R	R	R	R	R	R	R	R	R	R
“ <i>squarrosus</i>	143	R	R	R	R	R	R	R	R	R	R	R
“ <i>tectorum</i>	165	S100	S100	R	S100	S70	R	R	R	R	R	S100
<i>E. canadensis</i>	341	R	R	S100	R	R	R	S50	S100	S100	S100	R
“ <i>glauca</i>	342	S85	S100	S40	S	S100	S80	R	S100	S80
“ <i>sibiricus</i>	338	S20	S20	S100	S10	R	R	S75	R	S50	S50
<i>H. nodosum</i>	274	R	R	S100	R	R	R	S50	S100	S100	S100	R

was indicated in the 1938 experiments, *viz.*, that *E. canadensis* is susceptible to the head smut so prevalent in the native annual, *B. mollis*. The results of the 1939 inoculations further demonstrate also the virulence of collections M-C, M-L, M-I, and M-R on a few grasses of the *Hordeae*, as was indicated in 1938.

TYPES OF SYMPTOMS PRODUCED ON THE SAME HOST BY DIFFERENT COLLECTIONS
OF *USTILAGO BULLATA*

In 1938 and 1939 it was observed that various collections of *Ustilago bullata* produced different symptoms on those host species that are susceptible to several collections. Chief among these are *Bromus japonicus*, *Elymus canadensis*, *E. glaucus*, *E. sibiricus*, and *Hordeum nodosum*. Some collections were observed to produce varying percentages of infection on certain hosts, but the sori were decidedly latent in their development and inconspicuous, often never progressing beyond the size of a minute mass of smut spores concealed at the base of the floret. Yet, regardless of the very meager sporulation of the smut fungus in the inflorescence, the infected florets so far have always proved to be sterile. On other hosts the same collections produce well-developed sori, quite conspicuous by lavish sporulation. These two divergent types of expression of infection are the extremes, and various gradations occur between these. Furthermore, the host itself has to be considered, since on some even the most virulent of collections to which they are susceptible do not consume the entire florets, but form large bullate masses at the base of the florets.

Usually, in smut-infection studies, the percentage of infection is taken as the criterion of susceptibility or resistance and probably reliably so; but it is recognized in these investigations with *Ustilago bullata*, where different host species and genera have to be considered together with so many collections of the smut, that the type of infection is the real criterion of susceptibility. Percentage of infection may vary according to environmental conditions at the time of inoculation and the planting of the inoculated seed, but the type of infection resulting seems to be the true indicator of the degree of compatibility between host and parasite, just as it is in rust studies. In recording data from the 1938 and 1939 inoculations, it was necessary to designate in some manner the expression of susceptibility as well as percentages of infection. For this purpose 3 classes of reaction to collections of *U. bullata* were arbitrarily adopted: 1. Truly susceptible and compatible, as indicated by more or less well-developed, normal sori. 2. Susceptible, but not compatible, as indicated by sori more or less latent in their development. 3. Immune or highly resistant, as evidenced by the inability to develop even latent sori. This latter type represents a highly incompatible relationship between the grass and the smut fungus. These types of reaction were designated respectively as S, s, and R. These reactions and their variations seem rather comparable to the 5 types of reaction now universally recognized in cereal-rust research.

The appearance of the *S* and *s* types of infection and their gradations, as shown by the response of *Hordeum nodosum* and *Elymus sibiricus* to various collections of *Ustilago bullata*, are shown in figures 1 and 2 respectively.

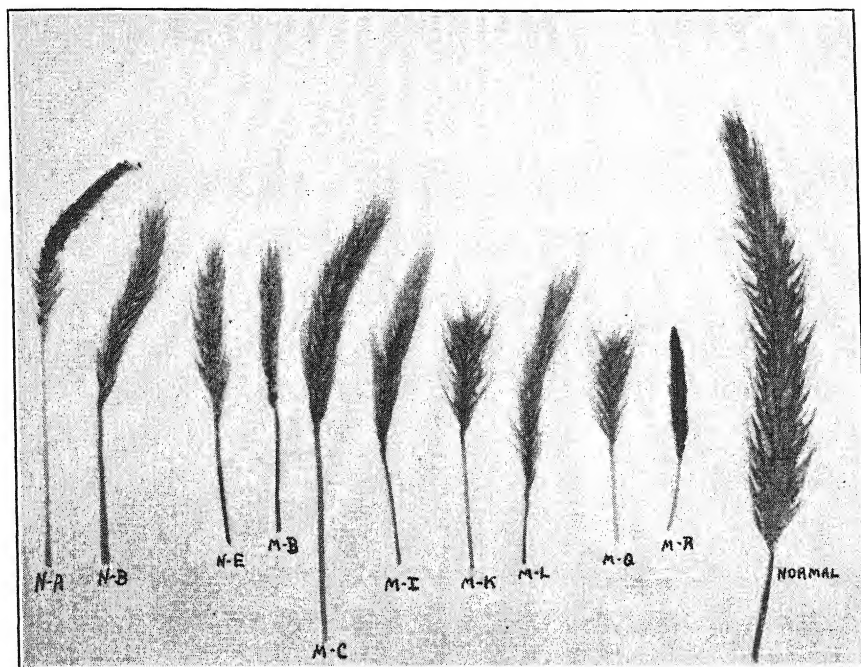


FIG. 1. Reaction of *Hordeum nodosum* (Acc. No. 274) to different collections of *Ustilago bullata* as follows: N-A from *Agropyron pauciflorum*; N-B from *A. cristatum*; N-E from *A. inerme*; M-B from *B. mollis*; M-C from *B. marginatus*; M-I from *B. catharticus*; M-K from *Bromus* sp.; M-L from *B. anomalus*; M-Q from *B. inermis*; M-R from *B. catharticus*. N-A, M-B, M-C, M-I, and M-R show the more compatible types of susceptibility (*S* type in text); the other collections show the more or less incompatible types (*s* type in text). Normal spike at right. Slightly less than nat. size.

PHYSIOLOGIC RACES IN *USTILAGO BULLATA*

From the results of the cross-inoculation experiments described above with 44 collections of *Ustilago bullata* from species of *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum* and *Sitanion*, it is possible to recognize at least 8 physiologic races of this smut fungus as shown in table 7 and described below.

Race 1

It is considered that collections (Table 1) N-A, N-F, N-J, N-O, N-S, R-A, R-B, R-E, and R-G probably all belong to one physiologic race, here designated as Race 1. The known host range of this race at the present time, on the basis of both inoculations and collections, includes *Agropyron caninum*, *A. pauciflorum*, *A. subsecundum*, *Elymus canadensis*, *E. glaucus*, *E. sibiricus*, *E. villosus*, *Hordeum brevisubulatum*, *H. jubatum*, *H. jubatum* var. *caespitosum*, *H. nodosum*, and *Sitanion hystrix*. Race 1 is common in

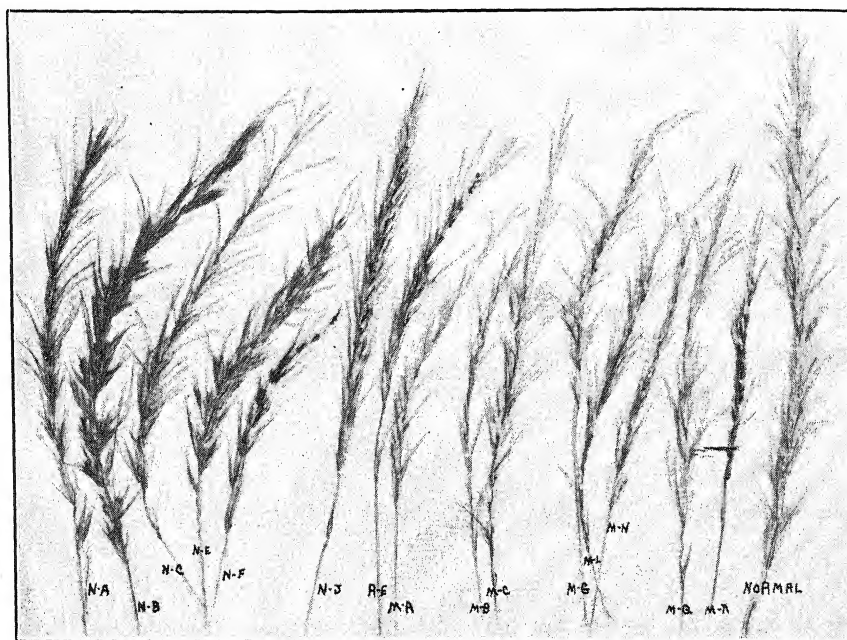


FIG. 2. Reaction of *Elymus sibiricus* (Acc. No. 338) to different collections of *U. bullata* as follows: N-A from *Agropyron pauciflorum*; N-B from *A. cristatum*; N-C from *A. dasystachyum*; N-E from *A. inerme*; N-F from *E. sibiricus*; N-J from *E. canadensis*; R-E from *Hordeum gussonianum*; M-A from *Bromus tectorum*; M-B from *B. mollis*; M-C from *B. marginatus*; M-G from *B. erectus*; M-L from *B. anomalus*; M-N from *B. sterilis*; M-Q from *B. inermis*; M-R from *B. catharticus*. N-A, N-F, N-J, R-E, M-C, and M-R show the more compatible types (*S* in text) of susceptibility, while the others show the more incompatible types (*s* in text). Normal spike on right. Approx. $\frac{1}{2}$ nat. size.

TABLE 7.—Differentiation of 8 physiologic races of *Ustilago bullata*

Differential species	F No. ^a	Other No. ^b	Race 1	Race 2	Race 3	Race 4	Race 5	Race 6	Race 7	Race 8
<i>Agropyron pauciflorum</i>	67	Wn. 279	S ^c	R	R	R	R	R	R	R
<i>Bromus brizaeformis</i>	245	W. 2578	R ^d	R	R	S	R	S	R	S
“ <i>carinatus</i>	239	W. 2725	R	R	R	R	S	R	R	R
“ <i>catharticus</i>	161		R	R	R	R	S	R	S	R
“ <i>hordeaceus</i>	155		R	R	R	s	R	R	R	S
“ <i>japonicus</i>	154		R	s ^e	R	S	R	S	R	R
“ <i>marginatus</i>	48	Wn. 439	R	R	R	R	S	R	S	R
“ <i>mollis</i>	194		R	R	R	S	R	R	R	R
“ <i>secalinus</i>	329		R	R	R	R	R	R	R	S
“ <i>tectorum</i>	165		R	S	R	R	R	R	R	R
<i>Elymus canadensis</i>	341	W. 2389	S	s	S	S	S	s	S	s
“ <i>glauca</i>	342	W. 1851	S	S	R	R	R	R	R	R
“ <i>sibiricus</i>	338	W. 225	S	s	R	R	S	R	S	R
<i>Hordeum nodosum</i>	274	W. 2723	S	s	S	R	S	R	S	R

^a F No. = Author's accession number.

^b W. No. = Accession number of the Soil Conservation Service.

Wn. No. = Accession number of the Wash. Agricultural Experiment Station.

^c S = Truly susceptible, as evidenced by more or less well-developed sori.

^d R = Highly resistant or immune, no evidence of infection.

^e s = Susceptible but incompatibly so, as characterized by more or less latent development of the sori.

the Northwest on *A. pauciflorum*, *H. nodosum*, and *H. jubatum*, and probably is the race of *U. bullata* destructive to slender wheatgrass in Canada (6, 13, 8).

Race 2

Race 2 includes collections N-B, N-C, N-E, N-G, N-L, N-M, N-N, N-P, N-Q, M-A, M-G, M-K, M-N, M-Q, and possibly M-X, and is by far the most prevalent of the races of *Ustilago bullata*. The known host range, on the basis of results of collections and inoculation experiments, includes *Agropyron caninum*, *A. cristatum*, *A. inerme*, *A. dasystachyum*, *Bromus tectorum*, *B. erectus*, *B. inermis*, *B. japonicus*, *B. sterilis*, *Elymus glaucus*, *E. junceus*, *E. sibiricus*, *Festuca idahoensis*, and *Hordeum nodosum*. The chief host is the common cheatgrass or downy brome, *Bromus tectorum*. Head smut on this grass is very prevalent throughout the "dryland" regions of the Pacific Northwest. It is an economically significant fact that this race, which is so effectively propagated on the widespread annual, *B. tectorum*, has such a long list of other more or less susceptible grasses, even in other genera. As the situation appears at present, however, the only species to be concerned about are *Elymus glaucus*, *E. junceus*, and *B. erectus*, which appear to be almost as susceptible as *Bromus tectorum*. Spikes of *Elymus glaucus* infected with race 2 are quite characteristic. The sori are rather well-developed but are entirely concealed within the palea and lemma, which are not consumed during sporulation. Each floret is expanded abnormally by the sorus within, giving the spike a more dense appearance. Usually, this is the only gross morphological distinction between the infected and non-infected spikes. Much the same effect has been observed on other grass species infected with race 2.

Race 3

Race 3 is represented by collection N-D only, which originated from a herbarium specimen of *Ustilago bullata* on *Agropyron scabrum* in Australia. Of the numerous species tested, only *Elymus canadensis*, *Hordeum nodosum*, and, occasionally, *Agropyron pauciflorum* seem at all susceptible.

Ustilago bullata was first described in 1855 on *Agropyron scabrum* from Australia by Berkeley (2); and it seems possible that the collection N-D, comprising race 3, might represent the original strain of *U. bullata*, which became the type of the species. The smut occurs also on *A. scabrum* in New Zealand (3). A morphologic expression of race 3 is seen in the tendency of the sori to consume the entire spikelets, or nearly so, and even extend down to the rachis. Illustrations of *U. bullata* on *A. scabrum* in Australia (11) and New Zealand (3) show the same type of sorus development.

Race 4

Race 4 of *Ustilago bullata* includes collections N-K, M-B, and M-O from *Elymus canadensis*, *Bromus mollis*, and *B. brizaeformis*, respectively. On the basis of inoculation experiments and collections this race will infect

Bromus brizaeformis, *B. mollis*, *B. brachystachys*, *B. commutatus*, *B. hordeaceous*, *B. japonicus*, *B. squarrosus*, and *Elymus canadensis*. Race 4 is quite prevalent (probably ranking second to race 2 on *B. tectorum*) in the Northwest on *B. mollis*. Certain strains of *Elymus canadensis*, a valuable species, seem to be quite susceptible, and a natural supply of inoculum usually is assured from the infected native stands of *B. mollis*.

Race 5

On the basis of the inoculation experiments with collections M-C, M-L, M-V, and M-W, it is considered that these represent another race of *Ustilago bullata*. The known host range includes *Agropyron caninum*, *Bromus marginatus*, *B. brevis*, *B. anomalus*, *B. purgans*, *B. polyanthus*, *B. carinatus*, *B. vulgaris*, *Elymus canadensis*, *E. sibiricus*, and *Hordeum nodosum*. This race is one of two causing head smut in native and planted stands of mountain brome-grass (cf. race 7). Smutted stands are common throughout the Northwest as well as other parts of the United States, and, even in the Hawaiian Islands. This race, together with race 7, is especially troublesome in nurseries where stands of *Bromus marginatus*, *B. polyanthus*, *B. catharticus*, and allied species are often 25 to 60 per cent or more infected. It is interesting to note that by artificial inoculation, *Elymus canadensis* is almost as susceptible as the above-mentioned grasses of the "mountain brome" group. These facts make race 5 one of the most important races of *U. bullata* in this country, since the grasses attacked are valuable forage grasses.

Race 6

Collections M-E, M-F, M-J, and M-U are considered as representing a sixth race of *Ustilago bullata*. The known host range includes *Bromus macrostachys*, *B. brizaeformis*, *B. japonicus*, and *B. squarrosus*. *Elymus canadensis* will sometimes show more or less infection. Race 6 seems to be close to race 4, but these two are easily separable by the susceptibility of *B. mollis* and *E. canadensis* to race 4.

Race 7

Collections M-I and M-R, both on *Bromus catharticus* are considered to represent another physiologic race of *Ustilago bullata*. The known host range, chiefly on the basis of inoculations, is *Agropyron caninum*, *B. brevis*, *B. catharticus*, *B. marginatus*, *Elymus canadensis*, *E. sibiricus*, and *Hordeum nodosum*. Race 7 is one of the most virulent of the known races of *U. bullata*. The spore production is exceptionally lavish. This race is very close to race 5, but the two are readily separable by the susceptibility of *B. carinatus* (F 239) to race 5. Collections M-T and M-Y are thought to belong to race 7, but need additional study to prove this definitely.

Race 8

Race 8 is represented by collection M-P from *Bromus secalinus* collected near Creswell, Oregon. The known hosts for this race include *Bromus*

brizaeformis, *B. hordeaceus*, *B. secalinus*, and *B. squarrosus*. *Elymus canadensis* sometimes shows latent infection.

Collections N-R and M-H, as seen from table 6, represent a distinct race. However, on the basis of their anomalous spore germination and perhaps other phases of their life history as well, it is recognized that these two collections may belong to some other species. For this reason, race numbers are not here given them. Their final disposition will be given in a later paper reporting on other collections of head smut.

DISCUSSION

As new collections of *Ustilago bullata* are studied in future years, new races probably will be found. The studies of the past several years do not include some species of grasses that have been reported as hosts to *Ustilago bullata*. Moreover, several species that have been reported have not proved susceptible to any of the 44 head-smut collections used in the present investigations, which facts indicate the existence of races not identified in these studies. These races should be sought out and investigated as to host range, virulence, and general biology.

The potency of certain of the races commonly found on native grasses makes these of considerable economic importance. Race 2, so very common in most of the "dryland" areas of the western United States, apparently can infect quite a number of species of *Agropyron*, *Elymus*, and *Festuca* in addition to other *Bromus* spp. Collections of head smut (all representing natural infection) from *Agropyron caninum*, *A. cristatum*, *A. dasystachyum*, *A. inerme*, *Bromus erectus*, *B. inermis*, *B. sterilis*, *Elymus glaucus*, *E. sibiricus*, *E. junceus*, and *Festuca idahoensis*, have all produced 60 to 100 per cent infection on *B. tectorum*, accompanied by lavish sporulation. Similarly, race 4, common on *B. mollis* in the same general regions where the *B. tectorum* smut is found, is virulent also on *Elymus canadensis*. Races 5 and 7, common throughout the Pacific Northwest and elsewhere on grasses of the "mountain brome" (*B. carinatus*) group, are virulent also on at least some accessions of *Agropyron caninum* and *Elymus canadensis* and to a lesser extent on *E. sibiricus* and *Hordeum nodosum*.

During the course of the inoculation experiments just described, the susceptibility of certain very promising grass species to one or more of the 8 races of *Ustilago bullata* has become apparent. *Agropyron pauciflorum* is quite susceptible to race 1, common in the Pacific Northwest on *Hordeum nodosum* and *H. jubatum*. *Bromus marginatus* is very susceptible to races 5 and 7, and smutty native stands of this grass are common, especially at the higher altitudes. Of outstanding general susceptibility, however, is *E. canadensis* which, as seen in table 7, is highly susceptible to 5 of the 8 physiologic races of *U. bullata*, and incompatibly susceptible to 3. *Elymus glaucus* is highly susceptible to two of the races, especially to race 2.

In considering the susceptibility of these important grass species it must be borne in mind that varietal response will no doubt prove to be a modifying

factor. It is highly improbable that all collections or accessions of *Elymus canadensis* will prove to be as susceptible as Acc. No. 341, nor will all accessions of *E. glaucus* prove as susceptible to race 2 as is Acc. No. 342. Perhaps, in final analysis, the most important basic fact to be gleaned from the results of these extensive inoculation experiments is that some of the most promising and favored grass species in a program of grass improvement and soil conservation are susceptible to head smut, and that, at least as far as these species are concerned, control measures are indicated either by seed treatment or the development of resistant strains. Some progress already has been made in this direction.

The head smut on *Elymus*, *Hordeum* and *Sitanion* spp. is still recognized by some as *Ustilago lorentziana*, that on *Bromus* spp. as *U. bromivora*, and that on *Agropyron* as *U. bullata*. In 1937 the writer (4) showed that, on the basis of comparative morphology, these 3 species blend and are inseparable. On this basis it was recommended that they all be considered as one species. The binominal *U. bullata* was suggested because of priority. Obviously, however, this concept has not been entirely accepted, as evidenced by the fact that recently Garrett (7) and Zundel (14) still recognize the 3 species as separate entities. Concerning *U. bullata*, Zundel states "This smut is near *Ustilago bromivora*, from which it differs in the hosts it attacks, its more evident appearance in the spikelets and the manner in which the epispore breaks up into the polar granular-verruculations (although *U. bromivora* has a very brittle epispore that breaks up into more uniformly scattered granular verruculations)." There is evidence that these differentiating characters are quite unreliable. The cross-inoculation studies described in the preceding pages appear to fully substantiate the arguments earlier presented by the writer (4) on the basis of comparative morphology, and further prove the fallacy of attempting to continue recognition of these 3 species as entities. In the first place they do not differ in the hosts attacked. Certain collections of head smut from *Hordeum*, *Elymus*, and *Sitanion*, which Zundel (14) lists as host genera for *U. lorentziana*, have been used to infect *Agropyron* spp. and *vice versa*. Other collections from *Elymus* spp. have proved pathogenic to certain *Bromus* spp. and *vice versa*. Certain collections on *Agropyron* spp. also have proved pathogenic to *Bromus* spp. and some collections from *Bromus* spp. have proved equally virulent on certain *Agropyron*, *Hordeum*, and *Elymus* spp. Therefore, even in addition to being similar morphologically (which alone should, without contention, constitute sufficient grounds for consolidation) the old species *U. bromivora*, *U. lorentziana*, and *U. bullata* are not even physiological entities.⁶

Any attempt to distinguish *Ustilago bullata* from *U. bromivora* by "its more evident appearance in the spikelets" (referring to *U. bullata*) is very

⁶ Since this paper was submitted for publication the writer has noted that very recently Mundkur (Kew Bull. No. 10, 1939) has stated that the merging of *Ustilago bromivora* and *U. lorentziana* with *U. bullata* represents the extreme extent of the view of the "neomorphologists," one of his objections being that the three species show a considerable degree of host specialization. It is hoped that the present results remove this objection.

apt to be unsuccessful because of the great variation and inconstancy of this character. Sometimes the smut on *Agropyron* is almost entirely concealed within the florets; other times the sori consume the entire spikelets and even extend down on to the rachis. Even on the same species in the field (as in a field of slender wheatgrass) these variations in the extent of development of the sori may be found. On the *Bromus* spp. the smut is rarely hidden within the florets, and rarely consumes the entire spikelets, being usually more or less conspicuous as bullate sori at the base of the individual florets. It would seem that this degree of "evident appearance in the spikelets" represents a host reaction as much as it does any quality of the smut fungus. For instance, race 7 of *U. bullata*, occurring on *Bromus* spp., produces even more conspicuous sori on certain other grasses, such as *Agropyron caninum*, *Elymus sibiricus*, and *Hordeum nodosum*, than it does on the *Bromus* spp.

Within the experience of the writer, the breaking up of the epispore "into the polar granular-verruculations," such that the polar regions are lighter, leaving a darker equatorial band around the spore, is no more characteristic of collections of head smut on *Agropyron* spp. than on *Hordeum* or *Bromus* spp. This darker, wide equatorial band has been observed in collections from all 3 genera and others in addition.

Garrett (7) states that his collections of smut on *Agropyron* species were identified as *Ustilago bullata* by Dr. Zundel. These included his Nos. 322 and 3076, both of which were "found on the stems and leaves and none in the inflorescence," which prompted Garrett (7) to ask: "Is this of any diagnostic importance?" And further, referring to the writer's proposal (4) to consider *U. lorentziana*, *U. bromivora*, and *U. bullata* as one composite species, he raises the question that perhaps the description of *U. bullata*, in the composite sense, should be broadened to include infection in the leaves, rather than merely in the spikelets, since all of his *Agropyron* smuts except one, referred by Zundel to *U. bullata*, sporulated in the leaves. He ends the discussion with the query, "If so, would not the composite species become even more composite?"

During the course of the past few years of extensive investigations of the head smut of grasses, the writer has examined scores of collections of this smut. He has made observations of large numbers of plants of several genera that had been artificially infected with most of these head-smut collections, and in only two instances has smut been observed in any part of the host plant other than in the inflorescence. No sporulation on any strictly vegetative parts has ever been observed in the thousands of infected plants matured in the greenhouse, where head smuts of cereals commonly sporulate in the flag leaf and leaf sheath.

Professor Garrett has kindly loaned me several of his grass-smut collections for examination, including Nos. 322 and 3571 on *Agropyron* identified as *Ustilago bullata* by Dr. Zundel. In the specimens received, sporulation of No. 322 had occurred in the leaves; of No. 3571 in both leaves and spikes. According to the writer's identification neither is *U. bullata*. The former

is *U. macrospora* Desmaz., and the latter is *U. striaeformis* (Westend.) Niessl.

On the basis of the above considerations it may logically be concluded that *Ustilago bullata* is typically a head smut and very rarely is found in vegetative parts of the host and then not exclusively, since it accompanies exceptionally lavish sporulation in the inflorescence. Therefore, the description of *U. bullata* should not be broadened to include leaf infection.

Liro (10) studied 3 collections of *Ustilago bromivora* (*U. bullata*), from *Bromus secalinus*, *B. mollis*, and *B. arvensis*. He found that the smut on any one of these was incapable of infecting the other two. In recognition of the physiologic specialization of these 3 collections he gave them all specific rank. The smut on *B. secalinus* was maintained as *U. bromivora*, since the type of this species had been described from smut on *B. secalinus*. The other two he named *U. bromi-mollis* and *U. bromi-arvensis*, after the hosts to which, within the limits of his experiments, they were specialized.

To follow what Liro has started would mean to recognize most of the races described in this paper as new species. The writer's races 4 and 8 may be the same as those Liro recognized as *Ustilago bromi-mollis* and *U. bromivora*, respectively. The remaining 6 races would be given some kind of binomial. However, the writer is not in sympathy with the nefarious practice of physiologic or biometric definition of species in fungi, and it seems preferable to consider these physiologic "species" as races.

SUMMARY

The results are presented of cross-inoculation experiments of 1936-39 with 44 collections of *Ustilago bullata* (including *U. bromivora* and *U. lorentziana*) from 36 species of *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum*, and *Sitanion*.

Eight physiologic races of *Ustilago bullata* have been recognized on the basis of the reaction of 14 differential species selected from the many species inoculated.

The heretofore supposed specialization of the smut from *Agropyron* and *Hordeum* spp. to grasses of the *Hordeae*, and of the smut from *Bromus* spp. to grasses of that genus has been found untenable. Certain collections from *Agropyron* and *Elymus* spp. were virulent on *Bromus* spp. and certain collections from *Bromus* spp. proved to be virulent also on *Agropyron*, *Elymus*, and *Hordeum* spp. Five of the 8 races have highly susceptible hosts in both the brome grasses and grasses of the *Hordeae*.

The most common race, which is widespread in the Pacific Northwest on the weedy annual cheatgrass, *Bromus tectorum*, has been found capable of infecting a number of species of *Agropyron*, *Elymus*, and *Festuca*, as well as a few other *Bromus* spp. Of the economic grass species, the most susceptible to this cheatgrass race of *Ustilago bullata* is *Elymus glaucus*. Another race common also in the Northwest on the weed *B. mollis* is quite virulent on *E. canadensis*, also an economic species.

The earlier recommendation of the writer that *Ustilago bullata*, *U. bromivora*, and *U. lorentziana* be consolidated because of similar morphology is now fully substantiated by the fact that the 3 species are not even separable on a host specialization basis.

The recognition of each physiologic race as a species, as started by Liro (10), is not considered desirable. Instead the 8 races are given numbers, in accordance with the common practice of designating physiologic races in the rusts and smuts.

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VARIATION IN HELMINTHOSPORIUM SATIVUM INDUCED BY A TOXIC SUBSTANCE PRODUCED BY BACILLUS MESPENTERICUS¹

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INTRODUCTION

There are numerous races of *Helminthosporium sativum* P. K. B., and in certain of these variations frequently occur. Some races of *H. sativum*

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do not mutate or vary readily under ordinary laboratory conditions, but may do so freely when subjected to special stimuli.

Experiments by many investigators indicate that the frequency of variations in many fungi can be altered profoundly by such environmental factors as light, temperature, hydrogen-ion concentration, nutrients, and the addition of certain salts and toxic substances to the substrate (1, 3, 8, 9, 10, 15). Some of these factors also may increase the frequency of variations in *Helminthosporium sativum* (4, 5, 6, 11, 12).

In recent years, numerous studies have been made on the mutual effect of the association of two different microorganisms (13, 16, 17). It has been demonstrated repeatedly that many bacteria and fungi are distinctly antibiotic toward certain other fungi (2, 7, 13). However, there is virtually no information on the effect of bacterial by-products on the frequency of variations in fungi. Recently, Christensen and Davies (6) found that the addition of a small amount of broth culture containing dead cells of *Bacillus mesentericus* (Flügge) Migula to ordinary potato-dextrose agar greatly increased the frequency of variations in certain races of *Helminthosporium sativum*. The present paper is a more detailed report on that subject.

MATERIALS AND METHODS

All the fungus cultures, including variants used in these experiments, were either monosporous, or, if nonsporulating, were hyphal-tip isolates. Most of the named species of bacteria used in these experiments were furnished by J. G. Leach, while the others were isolated by the writers from different sources.³ The latter group was purified in so far as possible by the dilution plate method.

Unless otherwise stated, the bacteria were grown on 1 per cent potato-dextrose broth, using about 300 g. of potatoes to a liter of broth. The basic solid medium for the growing of *Helminthosporium sativum* was usually the ordinary 1 per cent potato-dextrose agar. Most of the studies on frequency of variation were made in 90 mm. Petri dishes containing about 20 cc. of nutrient media. The number of such dishes used for each treatment varied considerably, but usually was 4 or 5.

EXPERIMENTAL RESULTS

In the fall of 1935, agar slants, contaminated with *Bacillus mesentericus* as a result of insufficient autoclaving, were reautoclaved, slanted and inoculated with numerous monosporous isolates of *Helminthosporium sativum*. In most of the tubes the fungi grew slowly and many sectors of different types developed, while the isolates growing on normal slants, not previously contaminated by bacteria, grew rapidly and produced no visible sectors.

From several tubes, in which the growth of *Helminthosporium sativum* was greatly suppressed, the agar was melted and mixed with fresh potato-dextrose agar in the ratio of 1:4, and then poured into Petri dishes. These

³ Three of the cultures belonging to the *Bacillus mesentericus* group were identified by Professor A. T. Henrici, Bacteriologist, University of Minnesota.

were inoculated with several races of *H. sativum*. The rate of growth of *H. sativum* was greatly inhibited and sectoring was common. The average diameter of the colonies on bacterium-staled⁴ medium was about 35–40 mm., while those of the control covered the Petri dish (approximately 90 mm.). Many of the colonies on the bacterium-staled medium developed from 3 to 10 sectors (Fig. 1, A), but no distinct sectors occurred in the control colonies.

LIVING BACTERIAL CULTURES

In order to test whether living cultures of *Bacillus mesentericus* would induce sectoring, *B. mesentericus* and *Helminthosporium sativum* were grown side by side in the same Petri dish containing about 20 cc. of 1 per cent potato-dextrose agar. The inocula were placed at various distances from each other, from virtually touching one another to as far apart as possible. In other cases the dish was inoculated first with one organism and then a day or two later with the other one. In every case *B. mesentericus* suppressed the growth of the *Helminthosporium* and the colonies of the two organisms never merged. In no case, although more than a hundred tests were made, did the fungus sector (Fig. 1, B.).

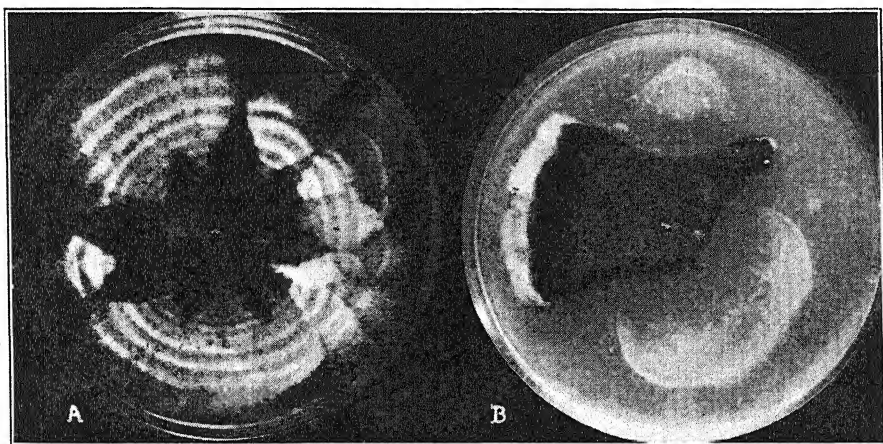


FIG. 1. *Helminthosporium sativum* race 1 growing in Petri dishes. A. Sectoring on potato-dextrose agar to which was added the toxic substance produced by *Bacillus mesentericus*. B. *Bacillus mesentericus* growing in a Petri dish with *H. sativum*. Although it suppressed the growth of the fungus, no sectors developed.

Other attempts were made to induce sectoring with living cultures of *Bacillus mesentericus*. For instance, potato broth containing a living culture of *B. mesentericus* was poured upon old and young colonies of two biotypes of *Helminthosporium sativum* race 1 and its variant 1–7, growing in Petri dishes. In still other cases, the central portion, including the agar, of 2- or 3-day-old colonies of several races of *H. sativum* was removed from the Petri dish and sufficient bacterial culture added to fill the hole in the

⁴“Bacterium-staled” is used in this paper to describe a medium in which *Bacillus mesentericus* alone, and not any other bacterium, has been grown.

agar. In both experiments there were no apparent increases in frequency of sectoring.

When the agar dishes on which these two organisms had grown in association were sterilized at 15 lb. pressure for 20 min. and reinoculated with *Helminthosporium sativum*, the growth of the fungus was much retarded and sectors developed abundantly. It is rather difficult to account for lack of sectoring when the bacterium and fungus were grown in association. Perhaps, as the fungus colony approached the bacterial colony, the concentration of toxic substance became too great and the fungus ceased to grow before the variants arose or before sectors became discernible. It is possible that two distinct effects are produced by the toxic substance, one a growth-inhibiting effect, and the other a sector inducing effect.

CONCENTRATION OF THE TOXIC SUBSTANCE

Only certain concentrations of the toxic substance produced by *Bacillus mesentericus* induced the development of variants. In general, potato-dextrose agar containing 2 or 3 per cent of the bacterium-staled broth gave the highest number of distinct sectors, and also the greatest number of variant types. Whenever the percentage of toxic substance became too high, the fungus either failed to grow or it grew so slowly that the presence of variants was not readily discernible. Usually, most variants arose when the growth rate of *Helminthosporium sativum* was restricted 35-50 per cent of the normal rate of growth on potato-dextrose agar.

Very weak concentrations of the sterile bacterium-staled broth of *Bacillus mesentericus* actually stimulated the vegetative growth of *Helminthosporium sativum* in liquid culture; whereas higher concentrations retarded growth and often increased sporulation. Also, very high concentrations retarded spore germination, caused abnormal development of germ tubes, and induced abnormal morphological changes in the vegetative mycelium.

Stability of the Toxic Substance

Sometimes severe suppression of growth would occur without any appreciable amount of sectoring; sometimes the same amount of bacterium-staled culture would not cause the same degree of inhibition. A series of tests was made to ascertain the possible cause for such fluctuations.

Erlenmeyer flasks containing 250 cc. of 1 per cent potato-dextrose broth were inoculated with a drop of a liquid culture of *Bacillus mesentericus*. These cultures were grown at room temperature for varying lengths of time and tested from 1 through 14 days of growth and some after several weeks of growth. On the basis of several tests, the production of toxic substance was found to increase up to 4 days. Several races of *Helminthosporium sativum* sectoried as frequently on agar containing 2 per cent bacterium-staled broth from a 4-day-old culture as from a 6-day-old culture or 3-week-old culture. Therefore, for stock material, potato broth cultures of *B. mesentericus* were sterilized at the end of 6 days and stored at room tem-

perature. These cultures retained their potency for many weeks, and, in two instances, for 4 months.

Repeated drying, wetting, freezing, and thawing did not seem to destroy the toxic principle. Broth cultures of *Bacillus mesentericus* were frozen and then thawed out on several successive times without reducing the potency of the toxic material. The bacterial broth was allowed to dry down in Petri dishes, and when the residue was restored to its original volume by the addition of water, it was as potent as the original solution. The pellicle from several broth cultures was removed and dried; it retained its sector-promoting power for several weeks in a dry condition.

The toxic substance produced by *Bacillus mesentericus* was apparently more or less thermostable. On several occasions nutrient agar containing the bacterium-staled broth was heated in the autoclave 2 or 3 times for about 20 minutes at 15 pounds pressure; other lots were heated continuously under pressure for more than an hour, yet there was no apparent destruction of the toxic substance. However, prolonged or repeated heating did reduce the strength of the toxic substance.

Effect of Type of Medium

The frequency of sectoring induced by the toxic substance produced by *Bacillus mesentericus* was definitely associated with the kind of medium on which the bacterium was grown and also with the basic medium used for the growth of *Helminthosporium sativum*. Other investigators (3, 4, 12) have shown that the type of medium influenced greatly the frequency of variation in certain fungi. The effects of adding bacterium-staled broth to eight kinds of media were ascertained, and the results were summarized in table 1. The

TABLE 1.—The effect of different media, with and without the addition of the toxic substance produced by *Bacillus mesentericus* on growth and sectoring of *Helminthosporium sativum* 1 and its variant 1-7

Medium	Size of colony in mm.				No. sectors per plate ^a			
	Race 1		Var. 1-7		Race 1		Var. 1-7	
	Ck.	Tox.	Ck.	Tox.	Ck.	Tox.	Ck.	Tox.
Beef peptone-dextrose agar	90	15	85	15	0.0	2.5	0.0	4.5
Brown's synthetic agar	70	12	60	10	0.0	0.2	0.0	1.4
Corn-meal agar	90	22	80	25	0.0	9.5	2.5	8.8
Glycerine agar	90	20	90	17	0.0	1.0	0.0	0.2
Malt agar	85	13	15	1.5	9.4	7.0
Oatmeal agar	90	25	90	27	0.0	10.2	0.0	7.8
Potato-dextrose agar (Acid)	27	27	3.6	4.3
Potato-dextrose agar (Basic)	50	45	27	30	1.0	0.8	1.5	0.4
Potato-dextrose agar (Neutral)	90	25	80	23	0.0	2.0	0.0	4.0

^a Results are based on averages of 4 or 5 colonies.

data indicated that malt, cornmeal, and oatmeal agars were the best media for production of variants in *H. sativum*, while Brown's synthetic medium and glycerine agar were the poorest. These media differed in their hydro-

gen-ion concentration, and this possibly accounted for differences in frequency of sectoring. Potato-dextrose agar, containing bacterium-staled broth and rendered distinctly acid by the addition of lactic acid, produced more variants than the same medium in a neutral or alkaline state (Table 1).

In general, the addition of a small amount of potassium hydroxide (KOH) to bacterium-staled broth destroyed its power to induce sectoring (Table 2). Several samples of bacterium-staled broth were made distinctly

TABLE 2.—The growth and frequency of sectoring in *Helminthosporium sativum* 1 and its variant 1-7 on potato-dextrose agar to which was added lactic acid, potassium hydroxide, toxic substance produced by *Bacillus mesentericus*, or combinations of these three

Treatment No.	Treatment and amount of material added to potato-dextrose agar	Size of Colony in mm. ^a		No. sectors per plate	
		Race 1	Var. 1-7	Race 1	Var. 1-7
1	Control	90	88	0.0	0.0
2	Lactic acid, 0.25 cc.	88	45	0.5	0.0
3	KOH, 0.20 cc.	80	76	0.3	0.0
4	Bacterial-staled broth, 2 cc.	50	47	8.0	7.8
5	Bacterial-staled broth, 2 cc.; KOH, 0.20 cc.	80	85	0.0	0.0
6	Bacterial-staled broth, 2 cc.; lactic acid, 0.25 cc.	60	35	6.8	4.0
7	Mixture of 2 cc. bacterial-staled broth and 0.20 cc. KOH	80	88	0.0	0.0
8	Mixture of 2 cc. bacterial-staled broth and 0.20 cc. KOH neutralized by lactic acid before addition to medium	90	88	0.0	0.0
9	Mixture of 2 cc. bacterial-staled broth and 0.25 cc. lactic acid, neutralized by KOH before addition to medium	70	68	7.0	4.2

^a Results are based on averages of 4 or 5 colonies.

alkaline by KOH and then brought back to the original hydrogen-ion concentration by means of lactic acid, but the toxic effect was not restored. In a similar manner, samples of broth from the same flask were made distinctly acid and then brought back to the original hydrogen-ion concentration by addition of KOH. In this case KOH did not affect the potency of toxic substance. The alkali was equally effective whether added before or after sterilization of the nutrient medium, or whether added directly to the broth or to the medium.

Races of fungi may be quite similar on one medium, but very different on others (4, 7). *Helminthosporium sativum* race 1 and its variant 1-7 were usually quite different culturally on ordinary potato-dextrose agar, but when grown on an alkaline medium, they appeared virtually identical. On acid media, however, they were decidedly different, variant 1-7 being much more sensitive to acid than its parent (Table 2). Therefore, it is possible that variants occurred on the alkaline medium, but their identities were not discernible.

Diffusion of Toxic Substance

Numerous observations indicated that the toxic substance produced by *Bacillus mesentericus* diffused out some distance (15–25 mm.) from the living bacterial colony grown on solid medium. The diffusibility of the substance was shown by covering $\frac{1}{2}$ of a Petri dish with bacterium-staled agar and the other half with nonstaled potato-dextrose agar. The dishes were then inoculated with *Helminthosporium sativum* at the junction of the two media. The rate of growth and also the shape of the colonies on two different but adjoining agars indicated that toxic material diffused slowly into the nonstaled agar. In another experiment, cubes of bacterium-staled agar, free from bacteria, were placed on potato-dextrose agar in Petri dishes about 25–30 mm. away from a young colony of *H. sativum*. The growth of the colony was inhibited in the region of the blocks. In still other tests, bacterium-staled agar obtained from regions around colonies of *B. mesentericus* was added to ordinary potato-dextrose agar. The growth of *H. sativum* on this agar was retarded and sectoring was rather common. All these tests indicated that the substance had diffused into the agar.

Filtration and Adsorption of Toxic Substance

The following experiments were made to determine whether the toxic substance was an exo- or endotoxin. Bacterium-staled broth was treated in different ways: one portion was sterilized by heat; a second was sterilized by heat and passed through a Berkefeld 3N filter; a third was filtered without the preliminary heat treatment; and the fourth was filtered and then re-sterilized by heat. When 2 per cent of these solutions were added to potato-dextrose agar, all the lots retarded growth (about 50 per cent) and stimulated the production of sectors. The results of the first filtration tests were not consistent. It was proved, however, that old used filters retained the toxic substance by its adsorption on some foreign material in the used filter. The passage of bacterial broth through new Berkefeld filters never decreased noticeably its potency, indicating that the sector promoting substance was an exotoxin.

The pellicle produced on broth cultures was distinctly more toxic to the growth of *Helminthosporium sativum* than the liquid portion of the same culture. This was not in perfect accord with the above filtration experiments, which indicated that filtrate free from bacterial cells was as potent as the broth culture containing the dead bacterial cells; however, this increased potency may have been due to the very great concentration of bacteria in the pellicle as compared to the concentration in the total broth culture.

The adsorption of the toxic substance on filters suggested the possibility of concentrating it on some solid material which could be stored and thus be available for use at any time. A few filtration experiments proved that the toxic substance was adsorbed by different materials: Wyojel clay, Wyodak clay, infusorial earth, and to some extent by animal charcoal. It was shown

by repeated filtration tests that 2 g. of infusorial earth would remove all the toxic substance from 100 cc. of bacterial broth. Consequently, it was possible to standardize the material, 0.2 g. of the toxic infusorial earth being equal in potency to 10 cc. of bacterium-staled broth. Many tests were made in which toxic infusorial earth was used instead of bacterium-staled broth, and the results were similar.

Inactivation of Toxic Substance by Microorganisms

It is known that some bacteria and fungi may be distinctly antibiotic to certain fungi in culture but not in the soil (2). There may be several reasons for this behavior. The substance that caused the antibiotic effect in culture may have been adsorbed on particles of soil and hence became too dilute to be effective. Laboratory experiments indicated that this actually happens. Several types of soil (sand, clay, and loam) were washed and dried and then used as filters. The results, especially with different lots of soils, were not always consistent. Even after the soils were washed they sometimes contained material that suppressed the growth of *Helminthosporium sativum*. In general, all three types of soil removed some of the toxic substance from the bacterium-staled broth, clay being by far the most efficient.

Some microorganisms may neutralize the toxic effects of others. *Coniothyrium* sp., *Dematium* sp., and *Botrytis cinerea* growing on bacterium-staled media destroyed or inactivated the toxic substance produced by *B. mesentericus* even when grown on the medium containing 10-20 per cent of bacterium-staled broth, while other species of fungi such as *Ustilago zeae* and *Acremoniella* sp. had no effect on the toxic substance. *Penicillium* sp. and *Cephalosporium* sp. inactivated the sector-promoting substance without destroying the growth retarding effect. The same was true of certain bacteria. Through an accidental contamination of *Bacillus mesentericus* with a coccus form of bacteria, it was noted that bacterium-staled broth, although retarding the growth of *H. sativum* did not induce sectoring. Experiments with broth staled by this mixed culture were repeated many times and several hundred cultures were involved. The results were nearly always the same, only an occasional colony developing one or more sectors. When, however, the culture was repurified it not only inhibited growth, but also induced the sectoring in *H. sativum* to the same extent as the original *B. mesentericus* culture did.

Specificity of the Toxic Substance for *Helminthosporium* spp.

The toxic substance produced by *Bacillus mesentericus* was rather specific in its action. Many species of fungi, both saprophytes and parasites, belonging to several genera, were grown on potato-dextrose agar containing 2 to 10 per cent of bacterium-staled broth. The toxic substance reduced growth to some extent in certain fungi, but it did not induce sectoring except possibly in one species of *Penicillium*. Potato-dextrose agar containing suffi-

cient bacterium-staled broth to reduce the growth of *Helminthosporium sativum* 50–60 per cent did not retard the growth or induce sectoring in other species of *Helminthosporium*. Thus, *H. tetramera* McKinney, *H. pedicellatum* Henry, and some unidentified species grew normally on 2–5 per cent bacterium-staled agar. Races of *H. sativum* and even variants derived from the same race responded differently to the toxic principle (Table 3 and Fig. 2).

The effect of adding bacterium-staled broth from bacteria other than *Bacillus mesentericus* was tried. Seven common bacterial plant pathogens, and about 30 cultures of unidentified bacteria isolated from various sources, were tested. None of these induced sectoring in *Helminthosporium sativum*, although a few did suppress its growth. One of the isolates stimulated sectoring to limited extent, in *Fusarium lini* Bolley (isolate 5), but it had no effect on *H. sativum* race 1 and its variant 1–7.

Eight isolates belonging to the *Bacillus mesentericus* group were tested for their production of products antibiotic to *Helminthosporium sativum*. Three of them were studied in considerable detail. Some of the isolates appeared different in cultural characters on liquid and solid media. Several tests indicated that the isolates also differed in their production of toxic substance promoting sectoring in *H. sativum*.

TABLE 3.—The effect of the toxic substance produced by *Bacillus mesentericus* on the growth and amount of sectoring occurring in six different races of *Helminthosporium sativum*

Race of <i>Helminthosporium</i>	Retardation in growth in percentages of check	Number sectors per plate ^a
1	75	7.0
101	75	5.6
102	66	0.2
104	66	1.3
106	56	5.6
107	75	7.6

^a Results are based on the averages of 4 or 5 colonies.

Effect of Temperature

Christensen (5) and Mitra (14) have shown that *Helminthosporium sativum* and other species of *Helminthosporium* sector most frequently near optimum temperatures for growth. Tests were made to see if the same relationship held when *H. sativum* was grown on potato-dextrose agar containing bacterium-staled broth (Table 4). The results, although preliminary, indicated that temperature was not nearly so important a factor when *H. sativum* was grown on bacterium-staled medium as when grown on ordinary media. Sectoring occurred at 15° C. on bacterium-staled medium, but not on ordinary potato-dextrose agar. In tests previously reported (5), no variations in *H. sativum* were obtained at 15° C. on nonstaled media.

The data in table 4 also indicated that, at temperatures 15° and 20° C., the growth of *Helminthosporium sativum* was retarded at least 50 per cent,

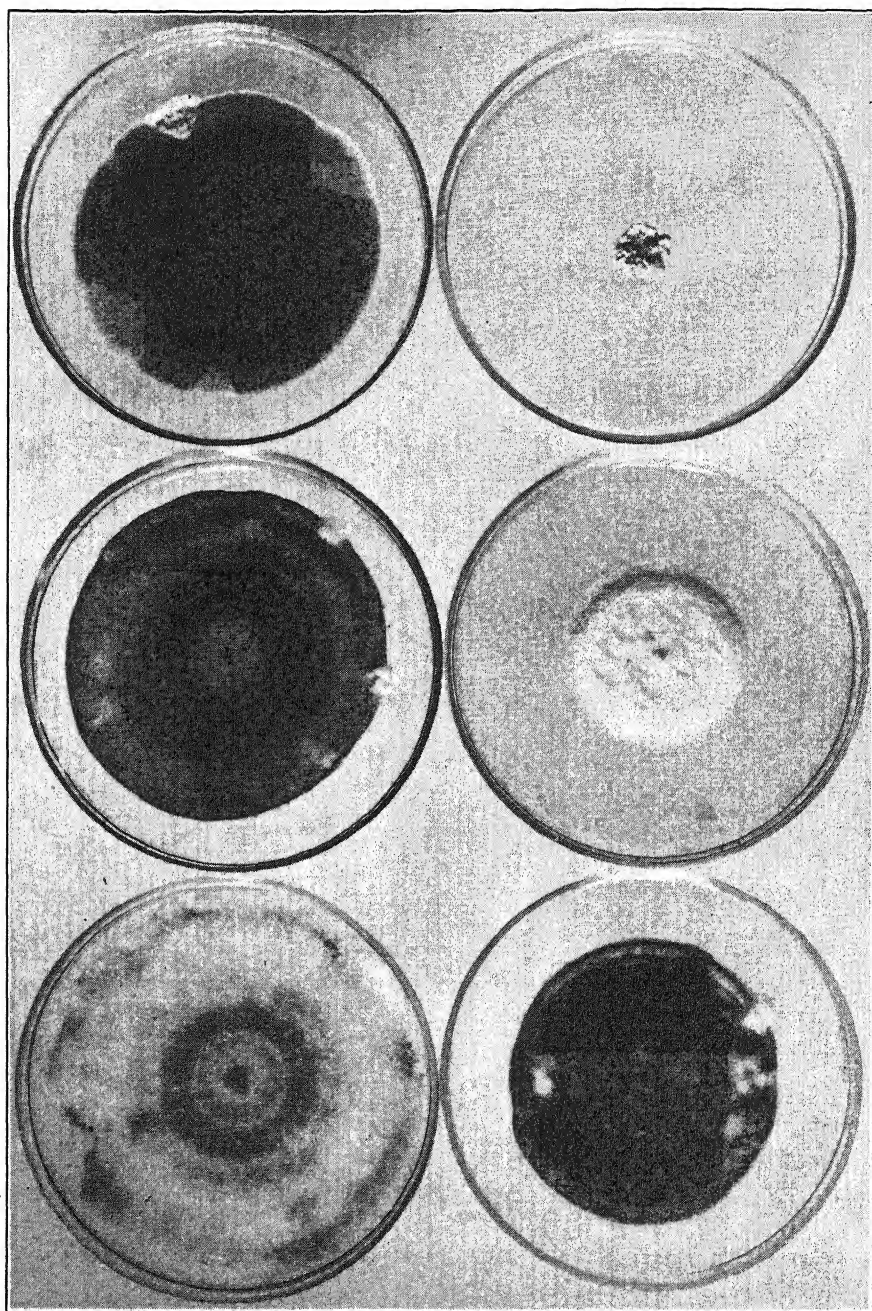


FIG. 2. The growth of 6 variants of *Helminthosporium sativum* 1 on potato-dextrose agar to which was added the toxic substance produced by *Bacillus mesentericus*. The growth of the parent colony, which is not shown, was approximately that of the variant in the lower right corner.

while at 25° and 30° C. it was retarded only 20 and 12 per cent, respectively. It is possible that a concentration of toxic substance great enough to reduce the growth at the higher temperature by 50 per cent would have induced a greater amount of sectoring.

Kinds of Variants

All the races of *Helminthosporium sativum* studied on potato-dextrose agar containing 2 per cent of the bacterium-staled broth gave rise to variants. Some produced many different variants. A detailed study was made of those produced by *H. sativum* race 1. At the beginning of these experiments, this race had been in culture for 17 years; it had been transferred several hundred times, and 4 successive single spores had been isolated. In the present tests, several hundred monosporous re-isolations were made and in a number of cases monosporous isolates in turn were made from them. These recently isolated subcultures of *H. sativum* race 1 were as stable as the parental type on ordinary potato-dextrose agar and sectoried as freely on potato-dextrose agar containing the bacterial toxic substance as did the original parent.

Forty distinct groups of variants were isolated from *Helminthosporium sativum* race 1. Within each group there were numerous biotypes with minor differences.

TABLE 4.—The effect of temperature on the growth and amount of sectoring of *Helminthosporium sativum* when grown on potato-dextrose agar to which was added toxic substance produced by *Bacillus mesentericus*

Temperature in degrees C.	Size of colony in mm. ^a		No. sectors per plate ^b	
	Ck.	Toxic substance	Ck.	Toxic substance
10	47	28	0.0	0.0
15	65	32	0.0	3.4
20	75	35	0.2	1.8
25	82	65	0.4	4.0
30	85	75	1.8	3.6

^a Colonies grown 16 days.

^b Results are based on the averages of 4 or 5 colonies.

The variants of *Helminthosporium sativum* race 1 differed from their parent in many characters: color, type and rate of growth, topography, kind and amount of pigment, abundance of sporulation, tolerance to adverse hydrogen-ion concentrations, reaction to dyes and other toxic materials, tendency to sector, pathogenicity, and to some extent morphology. Obviously, some variants differed not only in cultural characters but also in their physiological characters. If some of the extreme variants were found in nature, they would not be recognized ordinarily as *H. sativum*. For instance, some of the variants sporulated seldom or not at all in contrast to abundant sporulation of the parent; some developed a reddish pigment instead of the usual black, and others were albinos (Fig. 2 and 3).

Variants of *Helminthosporium sativum* race 1 reacted quite differently to

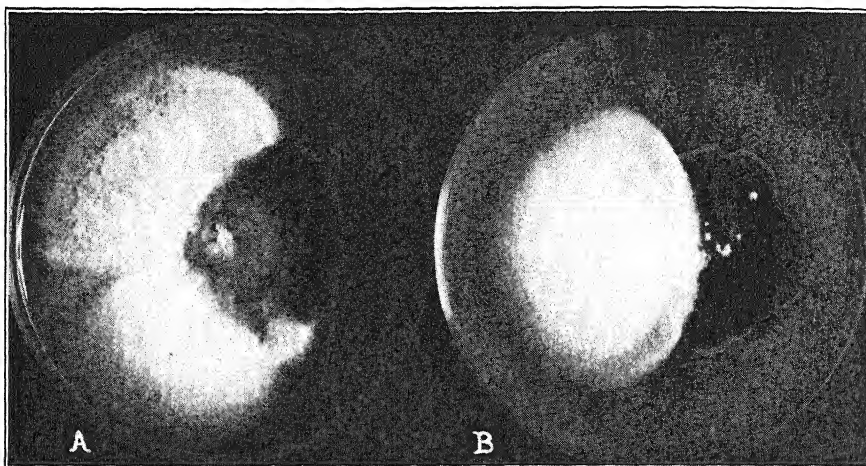


FIG. 3. *Helminthosporium sativum* 108 and its variants growing on potato-dextrose agar to which was added the toxic substance produced by *Bacillus mesentericus*. A. The parental colony (black) producing a variant (white), which grew partly over the parent. B. Parent (black) and variant (white), which were planted side by side; the parental colony was partly overgrown by the variant when photographed.

bacterium-staled broth. In fact, some variants were selected that grew at a perfectly normal rate on bacterium-staled agar at a concentration that suppressed the growth of the parent about 50 per cent (Figs. 2 and 3). There was a general tendency on the part of the workers to select variants possessing greater tolerance toward the toxic substance, for they grew faster and were usually the most definite and conspicuous and, consequently, most easily isolated. Similarly, when a stock culture of *Helminthosporium* was kept in test tubes on bacterium-staled agar, the original race was often lost and a variant more tolerant to the toxic substance than the parent perpetuated (Fig. 3).

When random samples of variants were isolated from bacterium-staled agar and also from other types of nutrient media, most of them had approximately the same degree of tolerance for bacterium-staled broth as their parent. However, among a few of these variants the range of tolerance to bacterial broth was very great, some were much more and some much less sensitive to the toxic substance than was their parent.

There were marked differences among the variants in their tendency to sector still further on potato-dextrose agar containing the staled broth of *Bacillus mesentericus* (Fig. 2). In general, most variants possess the ability to sector as frequently as their parent. None sectored more frequently than its parent; others sectored only rarely; and in some variants sectoring was not observed.

Additional proof that these induced changes were of a genetic nature rather than mere modifications of growth habit was indicated by their accompanying changes in parasitism. *Helminthosporium sativum* race 1 and 27 of its variants were tested for their virulence to Marquis and Mindum

wheats and to Velvet barley. These experiments were made as described by Christensen (4). Sterile soil was inoculated with a small amount of the culture grown on oat hulls. The tests were made in quadruplicate pots, each containing 25 seeds. In table 5 are recorded the reactions of Mindum to 12 variants chosen as representing the range of variation in parasitism. A few were more virulent to wheat and barley than was their parent; others were somewhat less virulent; and still others were virtually nonparasitic. In fact, the inoculum of certain variants actually stimulated the growth of the inoculated plants. Thus plants inoculated with variants 1-4 and 1-20 were decidedly more vigorous than were those inoculated with parental cultures and more vigorous than the controls. Variants 1-11 and 1-19 were much more virulent than their parent, *Helminthosporium sativum* race 1 (Table 5 and Fig. 4). The two nonvirulent variants (1-4 and 1-20) produced a few lesions on the foliage of the seedlings. Changes in parasitism in *H. sativum* induced by means of sectoring have been previously reported (5).

DISCUSSION

The present studies have shown clearly that environmental factors have a profound influence on the frequency of variation in *Helminthosporium sativum*. Broth, staled by *Bacillus mesentericus*, when sterilized and added

TABLE 5.—The susceptibility of Mindum wheat to *Helminthosporium sativum* race 1 and twelve of its induced variants

Variants of <i>H. sativum</i> 1	Percentages of plants			Green weight in grams ^a
	Emerged	Leaves with lesions	Stunted	
1	88	68	41	4.3
1- 2	92	22	13	5.4
1- 3	56	21	7	2.5
1- 4	76	26	11	6.7
1- 7	72	39	78	3.8
1-11	52	8	62	1.4
1-12	88	39	5	6.8
1-14	96	0	4	5.9
1-19	56	14	57	2.0
1-20	92	22	4	7.4
1-22	44	55	64	4.8
1-23	80	35	60	3.8
1-29	96	33	17	6.5
Control oat hulls not added	88	9	5	5.9
Control oat hulls added	92	4	0	6.9

^a Based on quadruplicate pots planted with 25 seeds each.

in small amount to ordinary potato-dextrose agar, suppressed the growth of *H. sativum* and stimulated the production of sectors. As might be expected, some of the variants isolated were better adapted than the parents to the new environment that induced their creation. Some grew perfectly well on a medium that was sufficiently toxic to reduce the parental type of growth by 50 per cent. Extensive tests with variants of *H. sativum* selected at random indicated that these were cases of natural selection, the survival of

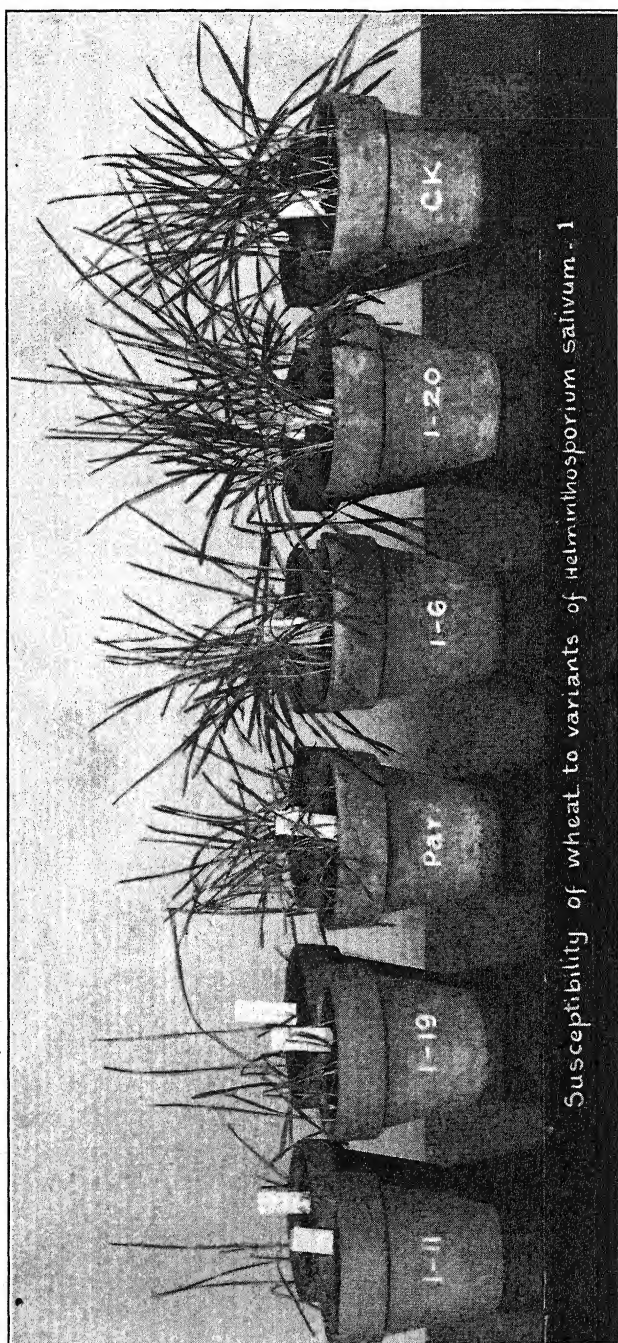


FIG. 4. The relative virulence of *Helminthosporium sativum* 1 and four of its variants induced by the toxic substance produced by *Bacillus mesentericus*, on *Minidum* wheat.

the variants best suited for this particular condition. Accordingly, mutation may result in a mixture of biotypes; the selective effect of the environment will perpetuate, to the exclusion or suppression of others, those variants most adapted to the conditions. Obviously, mutation may easily account for apparent loss of certain cultural characters and also account for a decrease or increase in the virulence of a pathogen.

The fact that sterilized bacterium-staled broth induces mutation in culture is not proof that *Bacillus mesentericus* also increases the frequency of variation in the soil or on the host. However, *B. mesentericus* is a common saprophyte of both soil and plant parts; thus it seems reasonable to assume that, under certain conditions, the proper association of two organisms might occur that could induce mutation in *Helminthosporium sativum*.

The reaction of several races of *Helminthosporium sativum* to substances produced by *Bacillus mesentericus* is an example of the high degree of specificity among microorganisms. A very minute quantity of the toxic substance produced by *B. mesentericus* induced frequent sectoring in *H. sativum*, but not in other fungi, including other species of *Helminthosporium*. Moreover, races of *H. sativum* and even of variants from the same race differed greatly in their response to the toxic substance. These studies indicate the complexity of the problem dealing with the interaction of microorganisms and the need of further investigations dealing with competition among microorganisms.

To the writers, it seems very improbable that the induced sectoring in *Helminthosporium sativum* race 1 was due to heterocaryosis. In the first place, race 1 was derived from several successive monosporous isolations and had been grown on artificial culture for nearly 20 years. Moreover, hundreds of monosporous isolates of this culture, made 18 years after its original isolation, were identical, yet all sectoried frequently when grown on potato-dextrose agar containing bacterium-staled broth. It is rather difficult to conceive of nuclei of different genetical constitution and without sexual attraction being held together for so long a time. Also, cytological studies have shown that the cells of conidiophores that give rise to conidia are usually uninucleate in *H. sativum* and, consequently, all the nuclei of the multinucleate spores are derived from a single nucleus. Hence, barring mutation, they are alike genetically. Therefore, it seems more reasonable to conclude that the genetic changes reported were due to mutation.

SUMMARY

Isolates of *Bacillus mesentericus*, when grown on artificial liquid or on solid media, produced a substance that suppressed growth, increased conidial production, inhibited or retarded germination, caused abnormal hyphal growth, and induced mutation in certain races of *Helminthosporium sativum*.

B. mesentericus did not induce sectoring when grown in close association with *H. sativum*, although it inhibited its growth.

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There was an optimum concentration of the toxic substance, which was conducive to the production of variants. The frequency of sectoring also was associated with certain types of medium and with relatively high temperatures.

The bacterial substance was thermostable, diffusible, passed through a Berkefeld filter, and was readily adsorbed on infusorial earth, withstood freezing and desiccation, and did not deteriorate appreciably on standing several months.

The toxic substance tolerated acid but was destroyed by alkali. Although the substance will withstand autoclaving for some time, prolonged heating or repeated heating tended to weaken its potency. The toxic substance also was destroyed or inactivated by certain bacteria and fungi.

Sterilized broth cultures of *B. mesentericus* when added to culture media suppressed the growth of many fungi, but did not increase the frequency of sectoring in any fungus, except *H. sativum* and possibly in one species of *Penicillium*. Even races and variants of *H. sativum* responded quite differently to the toxic substances.

A race of *H. sativum*, while growing on potato-dextrose agar containing the toxic substance produced by *B. mesentericus*, gave rise to numerous variants that differed in cultural characters, in general physiology, in pathogenicity, and, to some extent, in morphology.

By means of mutation, *H. sativum* adapted itself to a new environment, giving the appearance of increased tolerance to toxic substance produced by *B. mesentericus*.

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THE SUSCEPTIBILITY OF COTTON SEEDLINGS TO PHYMATOTRICHUM OMNIVORUM¹

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INTRODUCTION

In the extensive literature dealing with *Phymatotrichum* root rot of cotton and other plants, comparatively little information is to be found as to the reaction of very young cotton to the organism. Detailed observations are lacking for disease-seedling relationship under natural conditions in the field, and for experimentally induced infection under field or greenhouse conditions. This is rather surprising, since it is the common observation of root-rot investigators that young seedlings in the field rarely, if ever, succumb to the disease. Rogers (5) has expressed the general opinion:

"It is necessary that cotton plants become fairly well established before attacks by the fungus occur, since no seedlings have been found dying from the cotton root rot disease."

Although published accounts are meager, the writer has encountered much speculation among root-rot investigators as to the cause for the lack of infection of young seedlings in the field. The suggestion that soil temperatures are unfavorable for the organism when cotton is in the seedling stage is countered by the observation that alfalfa plants and other susceptible perennial hosts may be showing abundant symptoms of the disease at that time of the year. Furthermore, seed that may have failed to germinate until midsummer or newly produced seed results in seedlings apparently free of all symptoms of disease in the midst of actively progressing root-rot spots in the cotton field. Another theory, frequently discussed, is the possibility that the roots of the late-germinating seedlings may not have penetrated the soil sufficiently to have come in contact with the infective hyphae of the fungus. This might be particularly applicable under midsummer conditions at which time the upper soil layers are in an extremely dry condition and are unfavorable for the survival of the organism in those soil strata. It is, however, difficult to believe that the rootlets from some of the seedlings that are almost touching dying older plants have not come in contact with diseased roots or other infective material. Inasmuch as information upon the susceptibility or resistance of cotton seedlings is lacking in the literature, experiments were undertaken to determine the response of cotton seedlings to inoculations in the greenhouse.

¹ Cooperative investigations of the Texas Agricultural Experiment Station and the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Approved by the Texas Agricultural Experiment Station as Technical Contribution 600.

Since all evidence points to the fact that the root-rot organism will not survive the handling attendant to moving soil from infested fields to the greenhouse, it has been necessary to inoculate artificially the soil in greenhouse experiments. The efforts of other investigators in inoculation experiments are reviewed briefly by Streets (6). It appears that the most extensive greenhouse studies on infection of cotton plants were conducted by Neal and Ratliffe (4). They inoculated 48-day-old plants with infected alfalfa roots, pseudosclerotia from culture flasks, and with pure cultures of the organism grown on sterilized cotton roots. Infection was obtained with all three types of inoculum. They observed that the minimum time for the expression of aboveground symptoms was 14 days. While the true soil sclerotia of *Phymatotrichum omnivorum* had been discovered by King (1) shortly before Neal and Ratliffe initiated their inoculation experiments, sclerotia as such were not used by them, although they did use cultures derived from sclerotia. Within a short time after the discovery of sclerotia in laboratory cultures (1) and in nature (3) their pathogenicity was demonstrated in inoculation experiments reported independently by several investigators.

MATERIALS AND METHODS

During the period 1937-1939, experiments were conducted in the greenhouse on infection of cotton seedlings by the root-rot organism. As sources of inoculum, mycelial suspensions, agar cultures, naturally infected cotton roots, pseudosclerotia from culture flasks, and true soil sclerotia were employed. The seedlings were grown either on Houston black clay, Lufkin fine sandy loam, or Brazos river sand. The most consistent results were obtained with seedlings grown on Houston clay, inoculated with sclerotia. Unless otherwise specified this combination was used in the experiments hereinafter discussed. The sclerotia for the inoculation experiments were produced in flasks of moist Houston black clay to which slices of carrot were added before autoclaving. Following inoculation of the carrot-soil flasks with a culture of *Phymatotrichum omnivorum*, typical strands of the organism developed on the surface and among the soil particles of the culture; within 10 to 15 days numerous chains of sclerotia were formed. These were separated from the soil by washing, broken into small portions by agitation in water, and introduced into the soil near the roots of the seedlings to be tested, several sclerotia being used for each seedling.

In several of the experiments the seedlings were grown in 1-gallon glazed crocks filled with unwashed river sand or with Lufkin fine sandy loam. Considerable infection resulted from inoculations with sclerotia; but, in general, on Brazos river sand and on Lufkin fine sandy loam the inoculations were less satisfactory than those with Houston black clay soil.

Seed of Rogers Acala cotton was planted in deep metal cans, 18" x 8", containing Houston black clay soil. The stand of seedlings was usually thinned to 5 plants per can, although in several experiments as many as 10 plants were grown in each container. The maximum water-holding capacity

of the soil was 75 per cent of the oven-dry weight of the soil, as determined by the modified Hilgard method. Soil moistures were calculated, from known weights of soil and soil moistures at time of setting up the containers, to give the desired level or levels of soil moisture in the several experiments. The containers were brought to the required weight at approximately 3-day intervals during the period following inoculation. The heating system of the greenhouse was regulated to give an air temperature of 80–85° F., and the seedling containers, immersed in a water bath, maintained a soil temperature in or slightly below that range. This temperature range is known to be favorable for growth of the organism and the host.

After inoculation the seedlings were observed frequently for above-ground symptoms of infection, which usually consisted of wilting of the cotyledons followed almost immediately by wilting and collapse of the true leaves. With few exceptions death of the plant occurred 4 or 5 days later. Examination of the root systems of such plants showed typical buff-color strands of the root-rot organism on the surface of the seedling roots.

RESULTS

An experiment, factorial in design, was conducted for the purpose of determining the effects of (a) age of seedling and (b) soil moisture upon infection of cotton seedlings by the root-rot fungus. The seedlings were grown in containers at a common moisture condition until time of inoculation, at which time the moisture content of the soil was adjusted to 27, 36, or 45 per cent of the maximum water-holding capacity of the soil. The sclerotia were introduced into the soil near the roots of seedlings, 21, 28, or 36 days after planting of the seed. For each of the 9 treatments (age \times moisture combinations) 4 containers of seedlings were inoculated, with a stand of 10 seedlings per container.

The first evidence of aboveground symptoms of the disease was observed 8 days after inoculation of the 36-day-old seedlings at the highest moisture level, when 3 of the 4 replicates showed 1 wilted plant per container. By the 11th day symptoms were evident on 1 or more of the seedlings in all containers at the 2 upper moisture levels with inoculations made on both the 28- and the 36-day-old groups. Seedlings, 21 days old at time of inoculation, did not show symptoms of infection in all containers until the 15th day after inoculation. The lowest soil moisture, 27 per cent, was unfavorable for vigorous growth of the plants, and apparently was unfavorable for infection since but 3 of the 120 seedlings exposed to infection at this moisture level developed aboveground symptoms of disease within 24 days after inoculation.

Statistical analysis of the results showed that the two higher soil moisture groups did not differ significantly in the amount of disease and that both resulted in a significantly greater amount than occurred with the lower moisture. A significantly greater amount of disease was present in the oldest group of plants (36 days at time of inoculation) than in either of the

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younger age groups. Also the rate of development of symptoms was more rapid in the older plants.

It is generally recognized that moisture is an important factor in the development and spread of root rot in the field. Experimental work (5) has demonstrated that there are definite moisture limitations for the development of mycelial strands and for sclerotia production by the fungus. In the infection experiments reported here the lowest soil moisture was not favorable for infection and production of disease symptoms. Consequently, in subsequent experiments the moisture of the soil was maintained at about 40 per cent of its maximum water-holding capacity.

In order to obtain additional information on the response of very young seedlings, an experiment was conducted in which the sclerotia were placed in the soil at the time of planting the seed, and at 10, 20, and 30 days after planting. The contents of one flask of sclerotia were placed in the upper part of each of the soil containers, and the seed were planted approximately 3 inches above the layer of sclerotia. The inoculations at 10, 20, and 30 days after seeding were made by the usual method. The soil moisture level was at approximately 40 per cent. The results of the experiment are presented graphically in figure 1.

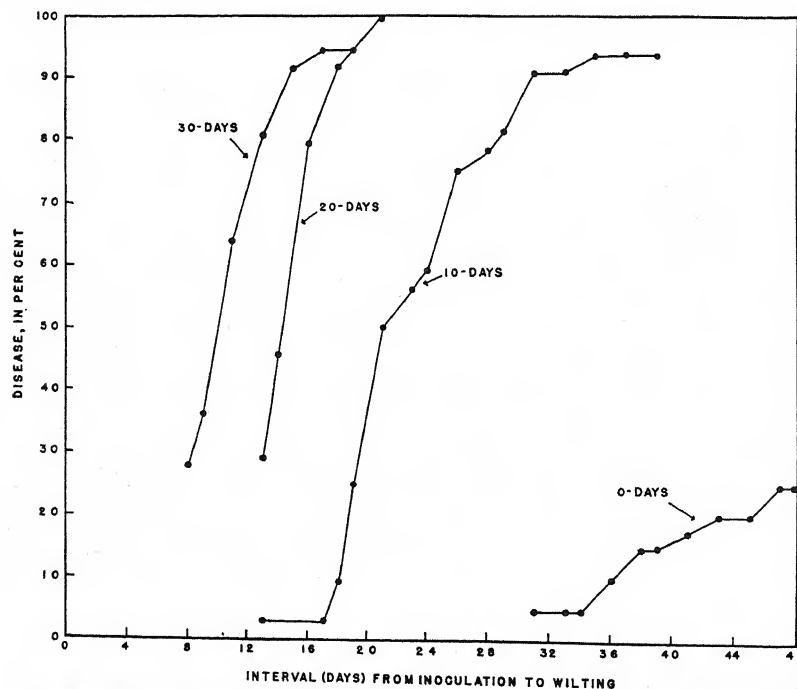


FIG. 1. Graphs showing the expression of aboveground symptoms of *Phymatotrichum* root rot in cotton seedlings inoculated at time of planting (0 days), and at 10, 20, and 30 days after planting.

The final percentage of disease was not appreciably different for the inoculations made 10, 20, or 30 days after planting, eventually reaching a

value above 90 per cent with these age groups. The minimum time required for expression of symptoms following inoculation was less with the 30-day age group than with the 20- or 10-day groups. The period required for complete expression of "susceptibility" was longer with the 10-day age group than with inoculations 20 or 30 days after planting. With the inoculation at time of planting, *i.e.*, sclerotia and seed planted together, the first above-ground symptoms of disease were observed 31 days after inoculation, and after 48 days only 25 per cent of the seedlings showed aboveground symptoms of infection. An examination of the graph for disease development in figure 1 reveals the scarcity of infection in seedlings that had been inoculated at the time of planting. There is a suggestion of a similar but much less pronounced delay for manifestation of symptoms in the next age group, with inoculation 10 days after planting.

It was confirmed by numerous additional experiments that infection rarely results when seed and sclerotia are planted together in soil or sand. However, periodic examination of the roots of the plants up to 6 weeks after inoculation frequently revealed the presence of the organism. In one experiment the seed and sclerotia were encased in paper cylinders approximately $1\frac{1}{2}$ in. in diameter to insure the contact of seedling roots and strands from the germinating sclerotium. After 18 days exposure only 2 plants were observed to be wilted and by 25 days an additional seedling showed symptoms of disease. No further expression of symptoms was evident after 45 days' exposure to the fungus in the population of 144 seedlings in the 49 cylinders.

These results demonstrate that when seed and sclerotia are placed together in the soil a very low percentage of the seedlings show aboveground symptoms, whereas, with inoculations at 10, 20, 30 or more days after planting, a high percentage of the seedlings become infected. This is illustrated in figure 1, which also shows that there is some evidence of susceptibility increasing with the age of the seedlings. That this period is definitely limited is demonstrated by the fact that the infection curves for the groups inoculated at 20 and at 30 days of age are almost parallel, and are nearly perpendicular. The relative resistance of seedlings inoculated at planting, and at the later dates is, however, entirely disproportionate to the differences in age alone. This suggests that, although the phenomena associated with age of the seedlings are of undoubted importance, the complete explanation must account also for the marked loss by the parasite of its capacity to cause infection resulting when the sclerotia are placed in the soil a few days "too soon."

With the methods employed in the inoculations it is believed that adequate opportunity for contact of host and parasite was provided, since the time required for germination of the seed and development of the radicle into and through the layer of sclerotia in the underlying soil should be coincident with germination of the sclerotia and subsequent development of strands through the soil. In such experiments conducted in glass con-

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ainers it has been possible to observe intermingling growth of the seedling roots and of the fungus strands, but, for some reason, the infection of the seedling tissues rarely progresses to the point of producing distinct lesions on the roots or the development of aboveground symptoms. In the literature but one reference has been found concerning the response of very young seedlings to the presence of sclerotia in the soil. McLean (2) reports:

"Pea, bean, and cotton seedlings grown in soil cultures of the fungus in the laboratory failed to become infected, although sclerotial masses in the culture were penetrated by the roots of the plants."

This observation is in accord with the results of the present investigation, and leads one to assume that the lack of infection is not due to failure of contact of the parasite and the host, but that it may be ascribed to some chemical or nutritional relationship between the host and the parasite, and that this relationship is somewhat altered as the seedling increases in age.

The observations by Watkins (9) were based upon the infection of cotton seedlings under pure culture conditions on a medium containing an abundant carbohydrate supply. It is interesting to note that with this method Watkins and Watkins (11) readily obtained infection of seedlings of even immune types of plants. Watkins has obtained further data (unpublished) which show that when seedlings and the fungus were placed on a noncarbohydrate substratum little or no infection of the seedling tissues resulted. In preparations involving seedling roots he found that starch grains were infrequently observed until the seedlings had attained the age of 3 or 4 weeks. Further evidence on differences in the composition of the very young cotton seedlings was made available through the courtesy of Paul J. Talley.² In unpublished experiments the changes in the percentages of fats, total sugars, and polysaccharides which occur during the sixteen days following planting of the seed in sand were investigated. Analysis during the early stages of germination shows a high oil content but a relatively low content of the various classes of carbohydrates. The results show a very rapid increase in the total sugars and polysaccharides at about 12 to 16 days after planting of the seed, and the sharp increase in these carbohydrates was believed to be due to the fact that the seedlings at that time were synthesizing and storing carbohydrates at a rate which exceeded their consumption.

From the above observations and previously discussed evidence it seems probable that differences in the carbohydrate content of the seedlings during germination and the early stages of growth have considerable influence upon the development of infection by the root-rot fungus. Furthermore, there are indications that if the seedlings are not sufficiently well supplied with carbohydrate reserves to meet the needs of the fungus for establishing itself in the host tissue, this lack may be overcome by artificial application. Conceivably this might be done by furnishing the inoculum with abundant carbohydrates so that large reserves would be built up, or it might be furnished during the incubation period. Some preliminary experiments were conducted to test both possibilities.

² Associate Professor of Biology, A. & M. College of Texas.

Talley and Blank (7) demonstrated the importance of various sources and amounts of carbohydrates in relation to growth responses of *Phymatotrichum omnivorum*. But it now seemed desirable to obtain some information on the amount of reserves that might be built up by the fungus and the importance of these reserves in relation to infection. Inoculum consisting of agar disks and adhering mycelium was taken from nutrient agar plates in which the carbohydrate supply, furnished by starch, was varied from 0 to 2 per cent. It is to be noted that the amount of mycelium in the disks used for inoculations necessarily varied considerably. When the inoculum was grown in the medium, either agar or liquid, to which $\frac{1}{4}$ to 2 per cent of corn starch had been added, the amount of growth was roughly proportional to the amount of carbohydrates in the medium. Without the addition of a carbohydrate, the amount of growth resulting from sclerotia was indeed scant, indicating that the carbohydrate reserve of the sclerotia was sufficient for initiating growth but not for stimulating continued growth, although it was sufficient to maintain life in the mycelium for several days. The disks were placed upon the radicles of seedlings germinating on water agar. No evidence of parasitism was observed when starch-free inoculum was used, while with the disks obtained from the $\frac{1}{4}$ per cent starch agar there was some tissue discoloration, but the establishment of infection was questionable. However, when the inoculum was derived from media containing only $\frac{1}{2}$ per cent of starch or more there resulted a rapid browning of the seedling tissues which was in immediate contact with the inoculum and there was no question as to the definite establishment of the fungus on the roots of the seedlings. Some question may be raised as to whether the carbohydrates necessary for the establishment of the fungus were stored in the mycelium, or were present in the small amount of agar or liquid medium incorporated in the inoculation disks. The fact, previously pointed out and to be noted below, that germinating sclerotia are able to infect older seedlings but do not have sufficient reserves to overcome the lack of carbohydrates in very young seedlings, casts some suspicion on the adhering nutritive medium. However this may be, it does seem important that the addition of carbohydrates to the inoculum, whether it results in reserves in the mycelium or is transferred with the mycelium and serves the fungus during the incubation period, overcomes the lack of carbohydrates in very young seedlings.

Further tests as to the importance of adding carbohydrates during the incubation period may be cited wherein host and parasite were cultured simultaneously on a series of media varying in their starch content. Germinating sclerotia were placed near germinating seedlings in Petri dishes containing nutrient agar, in which the carbohydrate content furnished by starch was varied from 0 to 2 per cent. The fungus failed to establish itself on seedlings on agar to which no carbohydrate had been added, although the hyphae from the germinating sclerotia came in contact with the radicles of the seedlings. The severity of infection increased rapidly as the starch content of the substratum was increased from a trace to 1 per cent. Inoculation

experiments were conducted also in soil to which $1\frac{1}{2}$ per cent starch, by moist weight of soil, was added or was omitted. As with the inoculations at time of planting, presented graphically in figure 1, infection was rarely obtained on very young seedlings when no starch was added to the soil. However, when a small amount of starch was added before autoclaving the soil, evidence of infection was observed on the roots of many of the seedlings within a week after the sprouted seed and germinating sclerotia were placed on the surface of the soil in the glass tubes. If one may assume that the relative resistance of the very young seedlings is due to the lack of sufficient carbohydrates for the establishment of the fungus in the host tissues, the preceding experiments suggest that this condition may be overcome by furnishing to the organism from external sources an adequate carbon supply. It is recognized that this type of experiment cannot furnish definite information as to cause of the apparent resistance of young seedlings but it is believed that the results strongly suggest the importance of the nutritional relationship between host and parasite.

Watkins and Watkins (10) pointed out that there was good evidence for believing that enzymes were involved in the processes of penetration and infection by *Phymatotrichum omnivorum*. It might then follow that adequate carbohydrate nutrition is required for such activity. Using this organism, Talley and Blank (8) showed the relationship of carbohydrates to the activity of several carbohydrases. Thus numerous observations and deductions relating to the nutrition of *P. omnivorum*, to methods of infection, and to apparently contradictory findings with respect to seedling susceptibility or immunity may, in reality, supplement each other in giving a logical explanation that would account for the immunity of cotton seedlings in the field, for the varying susceptibility under greenhouse conditions, complete susceptibility on nutrient agar, and for the laboratory observations of the intermingling of hyphae and roots with no evidence of parasitism. Nothing has been found by the writer to suggest that apparent seedling immunity is due to any inhibitory compound present in the seedling but disappearing with age. In summing up the present observation it would appear that if the seedling roots are old enough to contain available carbohydrates, and if the fungus is sufficiently active to initiate the first stages of infection, further enzymatic activity is stimulated so that subsequent penetration and establishment of the parasite in the host results. In very young seedlings, presumably low in available carbohydrates, the fungus attacks the roots only when supplementary carbon sources are supplied externally. If the above theory of carbohydrate relationship is supported by subsequent investigations, it may be that from the practical standpoint of disease control, cultural practices could be so modified that little undecomposed organic matter would be available in the soil during the late spring and early summer.

SUMMARY

Under favorable conditions of soil moisture and soil temperature the infection of cotton seedlings, resulting from inoculation with sclerotia of

Phymatotrichum omnivorum, occurred to a greater extent with older seedlings than with very young seedlings.

Inoculation at time of planting resulted in a longer incubation period and in a much lower percentage of disease than did inoculation at 10 days or more after germination of the cotton seed.

The accumulation of carbohydrates in the seedling tissues at approximately the time of a sharp increase in susceptibility to infection suggests the importance of the nutritional relationship between the host and parasite.

The addition of starch to autoclaved soil or to an agar substratum increases the infectivity of the organism to such an extent that seedlings are readily infected immediately following germination.

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INHERITANCE OF RESISTANCE TO CERCOSPORA ORYZAE IN RICE¹

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INTRODUCTION

Cercospora leaf spot, caused by *Cercospora oryzae* Miy., is the most serious fungus disease of Blue Rose rice in Louisiana. In the southern rice area,

¹ Joint contribution from Louisiana Agricultural Experiment Station and Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating.

² Assistant Plant Pathologist, Louisiana Agricultural Experiment Station, and Assistant Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, respectively.

which comprises parts of Louisiana, Arkansas, and Texas, Blue Rose is the most widely grown variety. In Louisiana it was grown on 355,944 acres in 1938 and 344,866 acres in 1939, or 73 and 72 per cent, respectively of the total rice acreage in these years.

Cercospora leaf spot on rice apparently has long been present in the United States. Metcalf (3) found a *Cercospora* associated with a disease on rice described as rust in South Carolina in 1906, but he thought the disease was of a physiological nature. On the other hand, Fulton (2) did not mention this disease in his bulletin, published in 1908, on rice diseases in Louisiana. It is probable, however, that as early as 1908-1910, Honduras, the principal commercial rice variety at that time, was very subject to *Cercospora* leaf spot.³ Beginning about 1912, Honduras was gradually supplanted by Blue Rose, due in part to the fact that Blue Rose at that time was relatively disease-free and continued to be so for a number of years. By 1920 (1) *Cercospora* had become rather common. Since then the fungus has so increased in the rice area that during the past 4 years, at least, the disease has appeared in epiphytotic form in every field of Blue Rose in Louisiana. In addition to the direct leaf-killing effect upon the plant, the premature dying of the leaves and leaf sheaths predisposes the plants to lodging. This has brought about a tendency in recent years for growers to harvest Blue Rose before it is well matured in order to circumvent lodging. A brief description of the *Cercospora* disease in this country has been given by Tullis (6). The disease occurs also in China, Japan, Philippine Islands, East Indies, West Indies, and Brazil (4, 7).

Control of *Cercospora* leaf spot is sought through the breeding of resistant varieties of the desired type. Certain commercial varieties are now highly resistant to the disease. In order to expedite the rice-improvement program in Louisiana, pathological investigations have been directed toward a study of (a) the nature of resistance, (b) the physiological specialization of the causal organism, and (c) the genetics of resistance. The present paper reports some of the results obtained in the genetic studies.

DISEASE RESISTANCE

Field observations, supported by artificial-inoculation studies, have shown that, on the basis of their reaction to *Cercospora oryzae*, rice varieties may be classified into 3 groups: highly susceptible, intermediate, and highly resistant.

The susceptible group includes Blue Rose, Early Prolific, Lady Wright, Edith, Honduras,⁴ and Carolina Gold.⁴ On leaves of these varieties the spots are large, 2 × 7-10 mm., and the incubation period is relatively short, 10-15 days during the summer months.

The highly resistant group includes Rexoro, Fortuna, Nira, Iola,⁴ C.I.

³ This statement is based on verbal communications from C. W. Edgerton, Plant Pathologist, Louisiana Agricultural Experiment Station, and J. M. Jenkins, Associate Agronomist and Superintendent of the Rice Experiment Station, Crowley, Louisiana, who made observations in these years.

⁴ Not grown commercially.

461,⁵ C.I. 4440, and a large number of introductions from various foreign countries. On these the leaf spots are small—1-4 mm. in length—and the incubation period is relatively long, 19-40 days during the summer months. As a result, no appreciable infection of these varieties builds up in commercial fields.

The intermediate group includes Caloro, Colusa, Vintula,⁴ Delitus, and Blue Rose selection 2854-3,⁴ which show variable reactions. In Caloro and selection 2854-3, this variable behavior has been shown (5) to be due to the fact that each of these varieties is susceptible to at least 1 pathogenic race of the fungus and highly resistant to others. Blue Rose also is partially resistant to 1 strain of the fungus. The fact that there are pathogenic races of the fungus may explain why Blue Rose remained relatively disease-free for a number of years after it displaced the susceptible Honduras as the principal variety. However, at present proof of this is lacking. The varieties listed above as highly resistant have proved to be resistant to all cultures of the fungus with which they have been tested. The disease reaction of 1 susceptible and 3 resistant varieties is shown in figure 1.

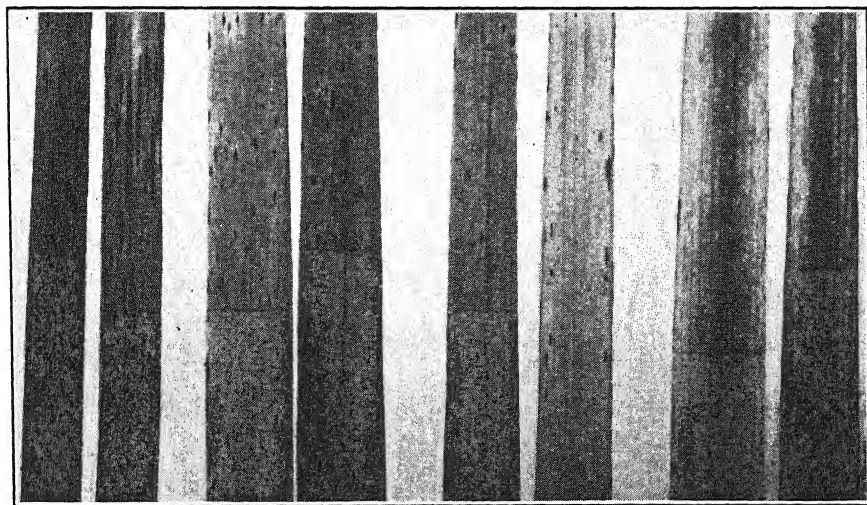


FIG. 1. *Cercospora* leaf spot on 4 varieties of rice, 36 days after inoculation. Left to right: Blue Rose, Fortuna, Nira, and Rexoro.

MATERIALS AND METHODS

During the growing seasons of 1936 and 1937, a number of crosses were made between resistant and susceptible varieties; and their progenies were subsequently tested for reaction to *Cercospora oryzae* under field and greenhouse conditions. The following crosses were made: Fortuna × Blue Rose; C.I. 461 × Early Prolific; C.I. 461 × Blue Rose; Blue Rose × C.I. 4440; C.I. 4440 × Blue Rose; and C.I. 4440 × selection 283A-10-1-1-3 (from Edith

⁵ C.I. refers to accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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× Fortuna). The F_1 plants, the F_2 populations, and in some crosses F_3 progenies, were tested in space-planted rows in the field at the Rice Experiment Station, Crowley, Louisiana, under conditions of high incidence of disease; also in pots in the greenhouse at Baton Rouge, where the plants were artificially inoculated. In some instances natural infection in the field was supplemented with artificial inoculation by atomizing the plants at sundown with a spore suspension of the fungus. In this way abundant infection was obtained. Inoculations in the greenhouse were made by atomizing plants with a spore suspension of the fungus and holding them in a moist chamber incubated at 28–32° C. for 24 to 48 hours. The plants were then moved outdoors. The length of the incubation period was obtained by this procedure.

EXPERIMENTAL RESULTS

In all crosses, the F_1 plants were resistant and the F_2 populations segregated (Fig. 2) for resistant and susceptible plants. The F_2 segregations of

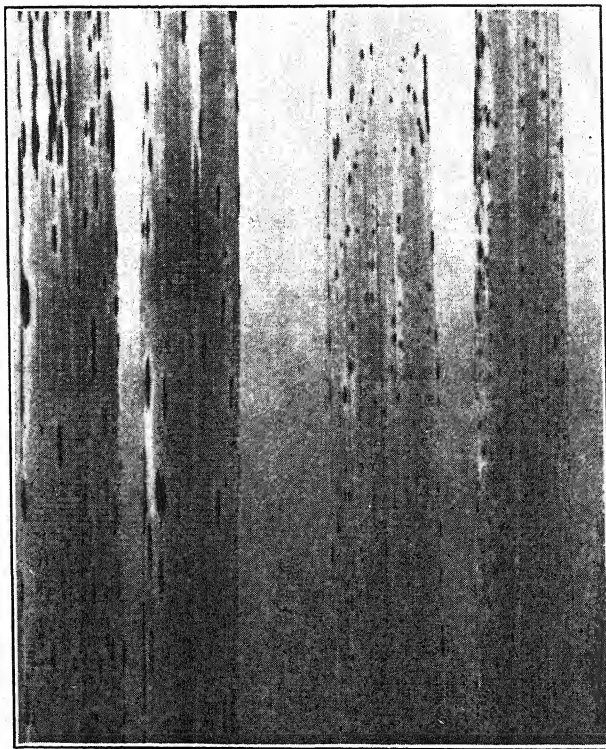


FIG. 2. Leaves from susceptible and resistant F_2 plants of a cross, C.I. 461 × Blue Rose, 30 days after inoculation.

all crosses are shown in table 1 and, with one exception, are in satisfactory agreement with the 3:1 ratio expected on the basis of a single pair of genes controlling resistance. The behavior of the exception, C.I. 4440 × selection

283A-10-1-1-3, can be explained by the fact that it was grown on land that had not been in rice for 17 years and natural infection was not very heavy. As this population was not artificially inoculated, no doubt many of the plants placed in the resistant class had escaped infection. All other crosses grown in this field received artificial inoculum and heavy infection resulted. The totals of the observed F_2 populations for the first 5 crosses listed in table 1 are in close agreement with the numbers expected on the basis of a ratio of 3 resistant plants to 1 susceptible plant. Chi square is 0.557 and P exceeds 0.40. When the F_2 progeny of the last cross are included in the totals, the fit is not so good. There is a deficiency in the susceptible class, presumably because of escape from infection. Chi square in this case is 3.591 and P is slightly greater than 0.05.

TABLE 1.—Segregation for reaction to *Cercospora oryzae* in the F_2 populations of 6 crosses under conditions of natural and artificial inoculation

Cross	Where tested ^a	Segregation in F_2 plants				Chi Square (3:1 ratio) ^b
		Year	Resistant	Susceptible	Total	
	<i>Louisiana</i>		No.	No.	No.	
Fortuna × Blue Rose	Baton Rouge	1938	58	16	74	0.450
	Baton Rouge	1939	71	16	87	2.027
C.I. 461 × Early Prolific.	Baton Rouge	1938	56	25	81	1.486
	Crowley	1939	389	113	502	1.660
C.I. 461 × Blue Rose	Baton Rouge	1939	78	24	102	0.118
	Crowley	1939	440	153	593	0.203
Blue Rose Selection × C.I. 4440	Crowley	1938	120	41	161	0.019
C.I. 4440 × Blue Rose	Crowley	1938	108	34	142	0.085
Total			1,320	422	1,742	0.557
C.I. 4440 × Selection 283A-10-1-1-3 ^c	Crowley	1939	348	83	431	7.580 ^d
Grand total ...			1,668	505	2,173	3.591

^a Artificially inoculated at Baton Rouge, and natural inoculum supplemented with cultures in some cases at Crowley.

^b Where Chi square = 3.841, $P = 0.05$. Higher values of Chi square indicate poor agreement with expected.

^c Selection 283A-10-1-1-3 is a selection from the cross: Edith × Fortuna.

^d Poor agreement probably due to escapes resulting from light infection in this plot.

It was possible in the greenhouse, where the populations were artificially inoculated, to compare the reaction of the plant with the incubation period. While the incubation periods differed from one time to the next, they were fairly constant for each series; that is, it was the same for all susceptible individuals and, likewise, for all resistant plants. For the latter, however, the incubation period was much longer than for the former. As an example: Inoculations were made July 18, 1930, on progenies of Fortuna × Blue Rose and C.I. 461 × Early Prolific; in both crosses the disease was observed on the 12th day in the susceptible segregates; whereas in the resistant plants there

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was no observable infection before the 26th day in the former cross and none before the 37th day in the latter cross. These periods corresponded to those for the respective parents. All the resistant plants in any one group showed maximum spotting within 2 or 3 days after the first spots appeared.

To check further the segregation, susceptible and resistant F_2 selections were made from 4 crosses in 1938, and the F_3 progenies were grown in 1939. These behaved as would be expected, if resistance were controlled by a single factor (Table 2). The susceptible F_2 plants were homozygous for susceptibility. Approximately $\frac{1}{3}$ of the resistant F_2 plants were homozygous for resistance and the other $\frac{2}{3}$ were heterozygous. The few discrepancies indicate errors in classifying F_2 plants, but these were no more than would be expected in making determinations on this kind of material. The F_3 rows were not space-planted, hence counts on F_3 segregation could not be made in the heterozygous resistant strains.

TABLE 2.—Breeding behavior of F_3 selections from four crosses

Cross	F_2 plants tested	Reaction to <i>Cercospora</i>	Behavior of the F_3 progeny
	No.		
Fortuna \times Blue Rose	8	Susceptible	All susceptible
	13	Resistant	4 resistant 8 segregating 1 susceptible
C.I. 461 \times Early Prolific	9	Susceptible	All susceptible
	19	Resistant	6 resistant 13 segregating
Blue Rose \times C.I. 4440	16	Susceptible	All susceptible
	14	Resistant	6 resistant 8 segregating
C.I. 4440 \times Blue Rose	15	Susceptible	13 susceptible 1 resistant 1 segregating
	13	Resistant	5 resistant 8 segregating

SUMMARY

The F_2 segregations in 6 crosses of resistant \times susceptible varieties were studied under conditions of natural infection and artificial inoculation with the common strain of *Cercospora oryzae*. In these crosses, under the conditions tested, resistance was due to a single dominant factor.

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A RAPID METHOD OF TESTING THE EFFECTS OF FUNGICIDES ON FUNGI IN CULTURE¹

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(Accepted for publication June 8, 1940)

A simple technique for the preliminary testing of fungicides has been devised that requires neither tedious microscopic work nor elaborate equipment. The test material is applied either as a dust or a spray to half of a fungus colony in a Petri dish. The fungicidal action is determined in a few days by observing the relative growth of the treated and nontreated portions.

The test fungus is prepared for treatment by transferring it to the center of a 9-cm. Petri dish containing an agar medium suitable for good growth. The culture is incubated until the mycelial mat is 2.5 to 3.0 cm. in diameter.

For testing fungicidal dusts, a desired quantity, e.g., 50 mg.,² of the material is placed on an 11-cm., fine-grain filter paper on which a circle the size of the bottom of a Petri dish has been drawn. The dust is spread evenly over the area of the circle with a camel-hair brush. A piece of aluminum foil about 8 by 12 cm. is then placed over half of the filter paper. The middle 9 cm. of one of the longer edges of this foil is turned up about 2 mm. The foil is placed with the turned-up edge next to and along the diameter of the drawn circle on the paper. As will be evident later, the foil limits the dust application to half of the culture, and the turned-up edge prevents the extraneous dust from sliding off. The cover is then removed from the Petri dish, and the bottom of the dish, containing the fungus colony, is inverted over the filter paper and foil, so that the edge of the plate coincides with the circle drawn on the paper. The entire outfit (filter paper, dust, foil, and bottom half of the Petri dish) is then inverted with one quick movement. Some of the fungicide will fall immediately upon the culture. That adhering to the half of the filter paper not covered by the foil may be dislodged by gently tapping this area of the paper with a pencil. Thus half of the fungus colony, with its corresponding portion of agar, is dusted with the fungicide, while the other half is protected with the aluminum foil. The paper and foil are then removed and the top portion of the Petri dish replaced.

A modification of the above procedure is used for applying sprays; it is used also in applying dusts, where the added complexity of treatment is be-

¹ The method is a modification of a technique suggested to the senior author by Dr. J. L. Horsfall, Entomologist, American Cyanamid and Chemical Corp., for use in studies supported by the above company. It is understood that similar procedures were used in the technical laboratories of that company as early as 1931.

² The size of the sample must necessarily be arbitrary in beginning studies with compounds of unknown toxicity, but may later be standardized.

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lieved to be warranted by the more quantitative and uniform application achieved. Half of the bottom portion of the Petri dish with the fungus colony to be treated is covered with a piece of tin with the edges bent and cut to fit over the rim of the dish. The culture is placed in the bottom of a spray tower,³ which is a celluloid cylinder 1 ft. wide and 3 ft. high. The desired quantity of the test material is introduced through an opening at the top of the tower by a spray gun. The latter consists of an air nozzle adjacent to the end of a small, calibrated glass tube containing the test material. The finely divided dust or spray particles settle uniformly upon the exposed culture. The plate is then removed and the cover replaced.

After application of the test material, by either procedure, a line is drawn across the bottom of the Petri dish, separating the treated and nontreated portions, which are designated as + and -, respectively. The outline of the colony also is marked on the bottom of the dish. The plates are incubated at optimum temperature for the fungus in question for the length of time it would take a normal colony to reach a diameter of 8.5 to 9.0 cm. Final readings are taken by holding a plate against a source of light and tracing on a sheet of paper held next to the plate, (1) the outline of the colony at the time of treatment, (2) the line demarking treated and nontreated halves (designating each), and (3) the final outline of the colony. Photographs (Fig. 1) may be taken when desired.

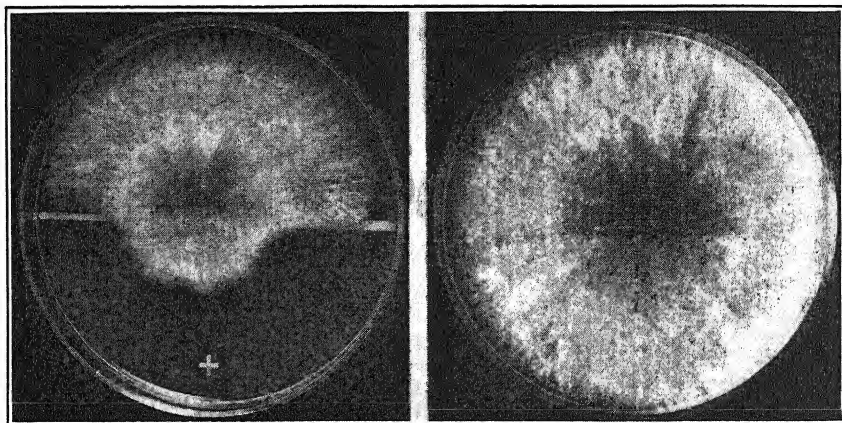


FIG. 1. Culture of *Diplodia zeae* (Schw.) Lev. treated with an organic-mercury dust. The marked outline of the colony at the time of treatment can not be seen through the mycelial mat. Nontreated culture on the right. (Photograph by Eugene Herrling.)

No difficulty with contamination has been encountered, although the plates are exposed to the air for a few seconds and the fungicides and equipment are not sterilized. If this difficulty should arise, the equipment may be sterilized and treatments made in a transfer chamber.

If the fungicide is effective, the growth of the mycelium is inhibited on

³ The spray tower was designed by H. A. Waters, Ohio State University Research Foundation, Columbus, Ohio.

the treated half of the plate, while normal growth (except as noted later) continues on the nontreated half. Figure 1 shows, on the left, a culture of *Diplodia zeae* (Schw.) Lev. treated with a fungicidal dust, using the technique involving direct application from filter paper as described. In this case, growth of the fungus was immediately stopped on the treated portion of the culture, while it continued uninhibited on the nontreated portion. Favorable fungicidal qualities of the dust are thus indicated. In testing various chemicals one finds all gradations from no inhibition of growth, through various stages of inhibition on the treated half, to complete inhibition of the entire colony. The latter occurs with very volatile or soluble toxic compounds. In such cases an untreated colony in another Petri dish may serve as a control. In any case, the use of a fungicide of known value in parallel series with the test material has proven an important guide.

There is always the possibility that the fungicides might react chemically with the medium itself, thus giving rise to inaccuracies in the results. It has not been determined whether or not any such reaction takes place, but the high correlation of results in the laboratory with those in the greenhouse and in the field, indicates that this discrepancy is within experimental error for the compounds tested. To meet this and other possible objections, investigators using this method would doubtless make such correlations in their own studies.

The method herein described provides an easy and rapid preliminary estimate of the fungicidal efficiency of materials, so that useless compounds may be discarded and the more promising ones subjected to further, more exacting trials. The writers have obtained satisfactory results with this method while examining large numbers of materials for their potentialities as seed disinfectants, or as protectant sprays or dusts.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

PHYTOPATHOLOGICAL NOTES

Potato Naturally Infected with California Aster Yellows.—Kunkel¹ failed to transmit the New York aster-yellows virus by means of the aster leaf hopper, *Macrosteles divisus* (Uhl.) [*Cicadula sexnotata* (Fall.)] to the following varieties of potatoes (*Solanum tuberosum*), which were either immune from or highly resistant to the disease: Irish Cobbler, Green Mountain, Bliss Triumph, and Spaulding Rose.

Severin and Haasis,² in 1934, reported that Bliss Triumph, White Rose, and potatoes grown from seeds were experimentally infected with the California aster-yellows virus by means of the aster leaf hopper, *Macrosteles divisus*. The symptoms were described and the incubation period of the

¹ Kunkel, L. O. Studies on aster yellows in some new host plants. Boyce Thompson Inst. Contrib. 3: 58-123. 1931.

² Severin, H. H. P., and F. A. Haasis. Transmission of California aster yellows to potato by *Cicadula divisa*. Hilgardia 8: 326-335. 1934.

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disease was determined. The virus was not recovered by means of previously noninfective aster leaf hoppers from infected potato plants nor from potato tubers obtained from plants showing symptoms of aster yellows.

A newly discovered leaf-hopper vector of the aster-yellows virus occurs in the canyons of the Montara Mountains, and many ornamental flowering plants grown in the coastal valleys, where the cut-flower trade is an important industry, are affected with aster yellows. This leaf hopper is a variety or physiological race of the aster leaf hopper. Specimens of the aster leaf hopper and the variety or physiological race were sent to E. P. Van Duzee, H. H. Dorst, P. W. Oman, and D. M. De Long; all agreed that the species could not be separated on morphological differences. The variety or physiological race has longer wing covers or elytrae than the aster leaf hopper, hence the former is designated as the long-winged aster leaf hopper and the latter as the short-winged aster leaf hopper. Long-winged and short-winged aster leaf hoppers will not cross or interbreed. Specimens of the short-winged aster leaf hopper were sent to H. H. Dorst by the writer³ and by Kunkel from New York, and all were determined as *Macrosteles divisus* (*Cicadula divisa* Uhl.).

A volunteer potato plant was collected on October 13, 1916, in one of the valleys of the Montara Mountains near Montara, showing purple, sessile, aerial tubers (Fig. 1) that grew from the axil of the leaves. Purple dwarfed leaves developed from the aerial tubers (Fig. 1).

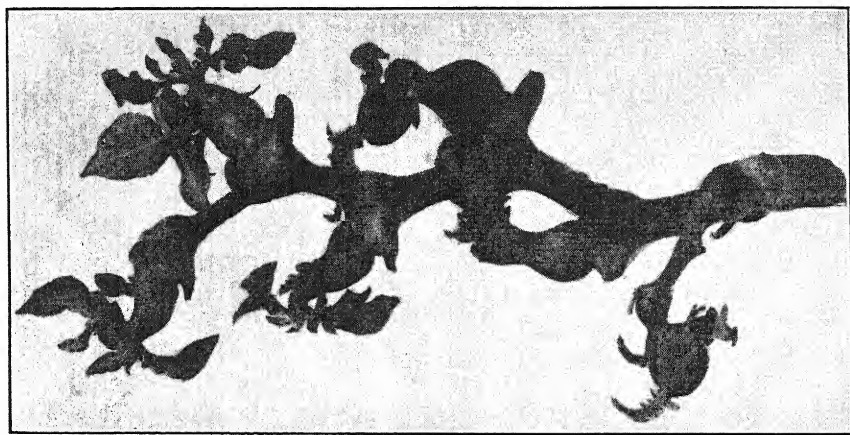


FIG. 1. Stem of potato plant infected with aster yellows with leaves removed showing sessile, aerial tubers growing from the buds at the nodes. Dwarfed leaves developed from the aerial tubers. (Valley in Montara Mountains near Montara, October 13, 1936.)

Two lots of 20 previously monoinfective long-winged aster leaf hoppers after feeding on the diseased potato for an average period of 4 days, were transferred and kept on 2 healthy aster plants that developed typical symp-

³ Severin, H. H. P. Experiments with the aster-yellows virus from several states. *Hilgardia* 8: 305-325. 1934.

toms of aster yellows. It is evident from this experiment that the potato was naturally infected with aster yellows.

Eight potato plants showing aerial tubers were collected near Colma, but previously noninfective long-winged and short-winged aster leaf hoppers, after feeding on the diseased plants, failed to transmit the aster-yellows virus to healthy asters. Nymphs and adults of the tomato psyllid, *Paratrioza cockerelli* (Sulc) were present on these potato plants and the cause of the disease was psyllid yellows. Plants grown from tubers from these 8 potato plants failed to show symptoms of psyllid yellows. Previously noninfective aster leaf hoppers failed to recover the aster-yellows virus from the plants grown from tubers and transmit it to healthy asters.—HENRY H. P. SEVERIN, California Agricultural Experiment Station, Berkeley, California.

Sodium Hypochlorite Shows Promise as a Seed Treatment.—This is a brief report on experiments that suggest practical possibilities for the use of sodium hypochlorite for controlling seed-borne diseases.

Although calcium hypochlorite has been employed for many years in surface-sterilizing seed for experimental purposes,¹ the writer has found no reference to the use of either calcium or sodium hypochlorite for practical seed-treatment purposes. Commercial preparations containing calcium or sodium hypochlorite are readily available and are not particularly expensive. In this laboratory, cotton seedlings and other diseased plant tissues are generally surface-sterilized with sodium hypochlorite solution preparatory to making tissue cultures. No washing between treatment and culturing is needed. Apparently, all or most of the active agent, chlorine, escapes by the time the causal organism begins growth.

Consequently, it was with considerable surprise that a residual germicidal effect was found to persist on cottonseed that had been surface-disinfected with sodium hypochlorite prior to inoculation, even after washing the seed for 2 hours in running water followed by 2 days' exposure to air. These observations led to greenhouse tests on treating cottonseed heavily infested with *Glomerella gossypii* (South.) Edg., the principal cause of damping-off. Figure 1 illustrates the type of results obtained with a commercial preparation of sodium hypochlorite.² Applications at the rate of 1½ to 4 oz. per bu. of seed approached in some cases the effectiveness of 5 per cent ethyl mercuric phosphate,³ which is recommended at present as standard treatment.⁴ In general, the results of the spray⁵ treatment were somewhat better than those obtained with the dust. It was thought that it might be possible to increase

¹ Wilson, J. K. Calcium hypochlorite as a seed sterilizer. *Am. Jour. Bot.* 2: 420-427. 1915.

² Sold as B-K.

³ Sold as New Improved Ceresan.

⁴ Haskell, R. J., and H. D. Barker. Cottonseed treatment. U.S.D.A. Leaflet No. 198. 1940.

⁵ A stock solution containing about 6% of available chlorine may be prepared by stirring 150 g. of B-K into 1000 cc. of water. After standing for several hours, the solution is decanted or filtered. It must be kept in the dark in well-stoppered bottles to avoid deterioration.

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the effectiveness of the hypochlorite powder by improving its dusting or adhesive qualities. This was not accomplished in an experiment in which bentonite was added to the hypochlorite powder.

Overdoses of sodium hypochlorite dust (1 lb. per bu.) appeared to cause but little or no injury to the seedlings. Application of the spray at concentrations comparable to this rate gave as good or better results than did the lighter dose illustrated in figure 1. However, the calcium hypochlorite which was used proved to be comparatively ineffective, even when applied at the rate of 1 lb. per bushel.

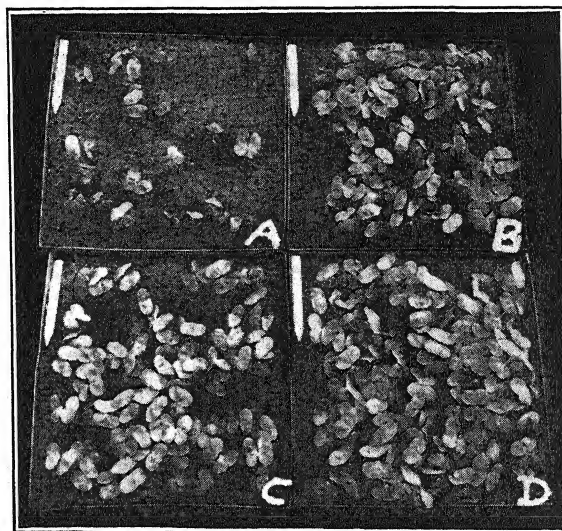


FIG. 1. Control of damping-off of cotton seedlings by seed treatment at the rate of $1\frac{1}{2}$ oz. per bushel. Variety Miller, heavily infested with *Glomerella gossypii*, planted in steamed river sand. A = nontreated; B = sodium hypochlorite dust; C = sodium hypochlorite spray; D = 5% ethyl mercuric phosphate.

One disadvantage of hypochlorites is their corrosiveness. Unlike chlorine gas,⁶ however, there would be no need for unusual equipment.

Although there appears to be little likelihood that sodium hypochlorite would be less expensive or more efficacious than the standard organic mercurials now recommended, this note is presented to suggest practical possibilities may exist where it is felt that the metallic germicides may be undesirable.—RICHARD WEINDLING, Bureau of Plant Industry, U. S. Dept. of Agriculture, South Carolina Experiment Station, Clemson, South Carolina.

Apparent Recovery of American Elms Inoculated with Ceratostomella ulmi.—In certain experimental studies it was noted that American elms (*Ulmus americana* L.), inoculated with *Ceratostomella ulmi* (Schwarz)

⁶ Leukel, R. W., and O. A. Nelson. Chlorine gas as a seed disinfectant. *Phytopath.* 29: 913-914. 1939.

Buisman, when not completely killed by the organism, often failed to express external symptoms of the Dutch elm disease the season following inoculation. This apparent recovery of diseased trees warranted further investigation. Accordingly, 115 trees approximately $\frac{3}{4}$ in. in diameter at the base, that had developed pronounced external symptoms shortly after inoculation in 1936, were set aside for this study. Check trees, intermingled with the inoculated group, never developed symptoms during the course of the experiment. In 1937, external symptoms of the disease developed in only 3 of the infected trees. Careful examination disclosed the fact that the organism had grown from the 1936 to the 1937 annual ring, and these 3 cases were not new infections. One of these 3 trees again developed external symptoms of the disease in 1938, but this was a "cross-over" of the organism from the 1937 to the 1938 annual ring. No further external evidence of disease has been shown by these 3 trees during subsequent seasons. With but these few exceptions, external symptoms of the disease resulting from the 1936 inoculations have not been apparent among this group of trees since the season of inoculation, and growth has been comparable to that of the nondiseased check trees.

Periodically, samples have been taken from each of the remaining 112 trees and cultured to determine whether *Ceratostomella ulmi* was still viable. Trees failing to yield the organism from the first sample were persistently resampled in the crown, trunk, and roots, and thorough attempts were made to recover the organism. Results are presented in table 1.

TABLE 1.—*Viability of Ceratostomella ulmi in 112 small American elms artificially infected in 1936, but not showing external symptoms of disease during subsequent years*

Date cultured	Trees yielding <i>C. ulmi</i>	
	Number	Per cent
January 1937	112	100.0
February 1938	110	98.2
January 1939	95	84.8
April 1940	82	73.2

The results indicate that the organism gradually died in some of the trees. In such trees recovery progressed from apparent to real. There was no spread of the disease from the nonrecovery symptomatic group to the checks.

On June 19, 1939, 10 of the trees from which *Ceratostomella ulmi* could not be isolated and 10 of the noninfected check trees were inoculated with a culture of *C. ulmi* that had just been isolated from the 1936 annual ring of an apparently recovered tree. External symptoms developed shortly after inoculation in all 10 trees of the former group and in 9 trees of the latter group. This experiment indicates that failure of the infected trees to develop external symptoms of the disease during seasons subsequent to inoculation was not attributable to any lack of pathogenicity of the organism or

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to any immunity acquired by the infected trees.—S. J. SMUCKER, Division of Forest Pathology, Bureau of Plant Industry, Morristown, New Jersey.

Importance of Verticillium as a Pathogen of Ornamental Plants.—During the past 2 years strains of *Verticillium* have been isolated by the writer from the following ornamental plants showing symptoms of hadromycosis: fall monkshood (*Aconitum Fischeri* var. *Wilsonii*), snapdragon (*Antirrhinum majus*), bachelor's button (*Centaurea cyanus*), florists and hardy chrysanthemums (*Chrysanthemum morifolium*), larkspur (*Delphinium ajacis*), common foxglove (*Digitalis purpurea*), sweet pea (*Lathyrus odoratus*), Oriental poppy (*Papaver orientale*), geranium (*Pelargonium hortorum*), annual phlox (*Phlox drummondii*), perennial phlox (*P. paniculata*), Chinese lantern (*Physalis alkekengi*), mignonette (*Reseda odorata*), greenhouse rose (*Rosa* sp.), salpiglossis (*Salpiglossis sinuata*), cineraria (*Senecio cruentus*), and African marigold (*Tagetes erecta*). All save larkspur and African marigold were cases of natural infection in commercial greenhouses or private gardens.

This appears to be the first report of *Verticillium* hadromycosis for fall monkshood, bachelor's button, larkspur, common foxglove, mignonette, salpiglossis and African marigold, and the first report in North America for annual and perennial phlox and Oriental poppy.

In inoculation experiments in which chrysanthemum strains of *Verticillium* were used, florists chrysanthemum, snapdragon, cineraria, annual phlox, and bachelor's button were found to be very infectible and intolerant,¹ larkspur was not very infectible but was quite intolerant, while African marigold, though very infectible, was quite tolerant. Plants of baby's-breath (*Gypsophila paniculata*), pot marigold (*Calendula officinalis*), garland chrysanthemum (*Chrysanthemum coronarium*), lupine (*Lupinus* sp.) and stock (*Matthiola incana*) did not become infected following either soil or wound inoculation in these experiments, though both lupine and stock have been reported susceptible to *Verticillium* in Europe. Additional ornamental susceptibles reported from Europe are monkshood (*Aconitum napellus*), aster (*Aster* sp.), China aster (*Callistephus chinensis*), royal bellflower (*Campanula macrantha*), clematis (*Clematis* sp.), dianthus (*Dianthus* sp.), foxglove (*Digitalis lanata*), fleabane (*Erigeron canadensis*), pearly everlasting flower (*Gnaphalium margaritaceum*), Maltese cross (*Lychnis chalcedonica*) and lavender-cotton (*Santolina chamaecyparissus*); while in this country dahlia (*Dahlia* sp.), California poppy (*Echscholtzia californica*), heliotrope (*Heliotropium* sp.), Shirley poppy (*Papaver rhoeas*) and veronica (*Veronica* sp.) have been listed.

Limited cross inoculation experiments failed to indicate any host specificity of the *Verticillium* strains studied, and it is the writer's opinion that isolates capable of infecting any one of the above ornamentals would also, under the proper conditions, prove infectious to most of the others.

¹ Terminology proposed by the Committee on Technical Words, American Phytopathological Society (Phytopath. 30: 361-368. 1940) is followed.

More exhaustive studies might show the existence of stable strains differing in their pathogenic relations, but at present the literature fails to support this view. The writer's experiments have shown that chrysanthemum strains are definitely pathogenic to snapdragon, annual phlox, bachelor's button, larkspur, African marigold, and cineraria, while workers in England have added China aster, stock, and sweet pea. Florists chrysanthemum and snapdragon, moreover, have been found by the writer to be susceptible to strains of *Verticillium* from chrysanthemum, snapdragon, geranium, salpiglossis, mignonette, cineraria, annual phlox and Chinese lantern.

Verticillium is present in most any planting of florists chrysanthemums in which a number of different varieties is included. Observations and experiments here summarized, therefore, emphasize the desirability of sterilizing chrysanthemum soil before replanting with any other ornamental crop, particularly such susceptible crops as snapdragon, bachelor's button, annual phlox, and cineraria. Although growers may under some conditions carry a susceptible crop in *Verticillium*-infested soil without serious wilt losses, the same crop may show very heavy losses under other conditions. Experiments are now in progress to determine some of the factors that may account for this variation in susceptibility or tolerance under greenhouse conditions.—A. W. DIMOCK, Cornell University.

*A Quick Method of Isolating Certain Phycomycetous Fungi from Soil.*¹—

As part of a study of seedling and root-rotting phycomycetous pathogens of small-seed legumes, barley, and corn, it became desirable to determine their presence in a large number of soil samples. None of the existing methods of isolating these fungi proved so convenient and effective as the one finally devised, namely, the planting of small portions of soil on nutrient-free agar. Details of the method are here presented, together with a record of the fungi isolated from infested Iowa soils.

Soil samples were collected from selected sites as follows: The soil surface was broken by means of a trowel or convenient tool. Immediately the mouth of a sterile test tube was thrust vertically to a depth of about an inch into the freshly exposed soil and the plug reinserted. In the laboratory the soil was removed from the tube with a small flamed scalpel and spread on the surface of a flamed cover glass resting on some convenient pedestal. A Petri dish containing two per cent nutrient-free agar was inverted over the soil on the cover slip and lowered until the uppermost soil touched the agar surface and adhered to it. The Petri dish was covered while inverted and kept in this position during incubation at 20° to 25° C. Single unbranched hyphal tips usually appeared at the edge of the soil mass in 24 to 48 hours. These were removed, together with a portion of agar, and placed on plain agar in another Petri dish. Such a procedure often resulted immediately in pure cultures. When a culture was contaminated with bacteria it was transferred

¹ Journal Paper No. J-754 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 432.

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to the under side of corn-meal agar, oat-meal agar, or nutrient-free agar in a Petri dish, as follows: A V-shape slit was cut in the agar. Then the V-shape portion was lifted with a long, flat, curved needle, a bit of the contaminated culture placed under it and the needle removed to allow the agar to settle as nearly as possible to its former position. The fungus usually grew up through the agar from the contaminating bacteria in 24 to 48 hours and could be transferred free of bacteria to a suitable medium.

It has been determined through observations of alfalfa plantings that *Pythium debaryanum* is less abundant in surface soil during mid or late summer after a protracted period of hot dry weather than during a period of moderate temperature and abundant moisture. Accordingly, isolation trials by the above method were made on different dates during the spring and summer of 1936 and 1937 to see if the results reflected a drop in the phycomycetous soil population.

Isolations from Clarion and Webster loams collected at Kanawha in northern Iowa and at Ames in April and May, 1936, yielded cultures that were assigned to the species, *Pythium debaryanum* Hesse, *P. ultimum* Trow, *P. rostratum* Butler, *P. echinulatum* Matthews, *P. pulchrum* v. Minden, *P. vexans* de Bary. In March, 1937, 50 isolations were made from Tama silt loam near Searsboro in Poweshiek County and Grundy silt loam near Oskaloosa in Mahaska County, Indianola in Warren County, and Lamoni in Decatur County, all in southern Iowa. Of these, 21 were *P. debaryanum*, 9 were *P. pulchrum* and 7 were *P. rostratum*. The other 13 were phycomycetes, but were not identified.

On July 3, 1937, 10 isolates were taken from soil in a corfield on Webster silty clay loam southeast of Ames. Six of these were *Pythium rostratum*, 3 were phycomycetes, which were not identified, and one a species of *Fusarium*. From July 23 to 27, 1937, 50 isolates were taken from soil samples collected at 2-3-inch depths in cornfields on Wabash silty clay loam, Clarion loam, Webster silty clay loam, Muscatine silt loam, and Tama silt loam. Only 1 phycomycete was isolated. It was identified as *P. ultimum*. On 4 other plates slow-growing phycomycetous hyphae were mixed with *Fusarium*-like growth, but it was impossible to grow the phycomycetes in pure culture and identify them.

Using this method it was seen that *Pythium* species were prevalent in the spring and were rare in late July, confirming what earlier had been in isolation studies.—CLIFFORD H. MEREDITH, Glenleigh Laboratory, Highgate P. O., Jamaica, B. W. I.

CORRECTION

In a paper by Gerold Stahel, entitled *Corticium areolatum*, the cause of the areolate leaf spot of citrus (Phytopath. 30: 119-130. 1940), line 1, paragraph 5, p. 119, change *Leptosphaeria* leaf-spot fungus to *Corticium* leaf-spot fungus.

NOTICE TO MEMBERS

Recognizing that members of the American Phytopathological Society in Canada and the United States receive material, as well as intangible, benefits from the status of their publication, PHYTOPATHOLOGY, as an international journal in this branch of science, it is to their interest to preserve as far as possible the Journal's international following, even in times when cooperation between nations has become a tragic mockery. In such times as these, many of our foreign members find it impossible to maintain their subscriptions. A number of cancellations have been received; more are expected with the beginning of 1941. This threatens the international status of our Journal, and may appreciably curtail the Society's income.

It is, therefore, suggested that our American membership, especially in peaceful, prosperous, and generous United States, accept this as an opportunity to aid in preserving their Journal's circulation and at the same time of extending their good will to hard-pressed colleagues by each contributing a modest sum to continue these foreign subscriptions. This can be done in either of two ways: (1) Individual members or a group of colleagues may wish to assume the full cost of a particular foreign subscription, thus putting the transaction on a personal basis; (2) others may wish to contribute a fractional amount of a full subscription to be pooled by the Business Manager with other contributions and used at his discretion in extending foreign subscriptions. If those contributing according to the latter plan wish to make reservations regarding the manner in which their donation is used, their wishes will be honored. In any event the Business Manager stands ready to facilitate this project of good will. Your cooperation will be welcomed.

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